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IOWA STATE COLLEGE  
**JOURNAL OF SCIENCE**

*A Quarterly of Research*



VOL. XIV  
1939-1940

PUBLISHED BY  
COLLEGIATE PRESS, INC.  
IOWA STATE COLLEGE  
AMES, IOWA

IOWA STATE COLLEGE

# JOURNAL OF SCIENCE

Published on the first day of October, January, April, and July

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All remittances should be addressed to Collegiate Press, Inc., Iowa State College, Ames, Iowa.

Single Copies: One Dollar. Annual Subscription: Three Dollars; in Canada, Three Dollars and Twenty-five Cents; Foreign, Three Dollars and Fifty Cents.

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Entered as second-class matter January 16, 1935, at the postoffice at Ames, Iowa, under the act of March 3, 1879.



# Vol. XIV, No. 1, October, 1939

|                                    |   |
|------------------------------------|---|
| Abstracts of Doctoral Theses ..... | 1 |
|------------------------------------|---|

# Vol. XIV, No. 2, January, 1940

|  |     |
|--|-----|
| Circulation of Hemolymph in the Wings of the Cockroach, <i>Blattella germanica</i> L. I. In Normal Wings. STEWART CLARE and OSCAR E. TAUBER .....  | 107 |
| Fungi Associated with Tree Cankers in Iowa. II. <i>Diaporthe</i> , <i>Apioporthes</i> , <i>Cryptodiaporthe</i> , <i>Pseudovalsa</i> and Their Related Conidial Forms. JOSEPH C. GILMAN and G. L. McNEW ..... | 129 |
| Preliminary Studies on the Comparative Value of Some Sprays and Dusts in Potato Insect Control. H. C. MANIS and ILA LEFFERT .....  | 155 |
| Examination of Ice Cream with the Burri Smear Culture Technic. H. F. LONG and B. W. HAMMER .....   | 163 |
| Preparation of an Active Juice from Bacteria. W. P. WIGGERT, M. SILVERMAN, M. F. UTTER and C. H. WERKMAN .....   | 179 |
| Description of a Dextro-Lactic Acid Forming Organism of the Genus <i>Bacillus</i> . A. A. ANDERSEN and C. H. WERKMAN.....  | 187 |
| Effect of Low Temperatures on the Intensity of Fluorescence. J. E. DINGER and WILLIAM KUNERTH .....  | 195 |
| A Supplement to the Catalogue of Iowa Plants in the Iowa State College Herbarium. ADA HAYDEN .....   | 199 |

# Vol. XIV, No. 3, April, 1940

|   |     |
|---|-----|
| The Efficacy of Ultra-violet Light Sources in Killing Bacteria Suspended in air. B. A. WHISLER.....   | 215 |
| Anaerobic Decomposition and Gasification of Cornstalks by Thermophiles. G. H. NELSON, R. P. STRAKA, and MAX LEVINE .....                                | 233 |
| The Dissimilation of Phosphoglyceric Acid and Hexosediphosphate by <i>Aerobacter indologenes</i> . R. W. STONE, M. N. MICKELSON and C. H. WERKMAN ..... | 253 |
| The General Differential Operator. FRED ROBERTSON.....  | 261 |
| Insects as Vectors of Yellow Dwarf, a Virus Disease of Onions. H. D. TATE .....   | 267 |
| Stresses in an Orthotropic Elastic Layer. R. H. TRIPP AND D. L. HOLL..  | 295 |
| Effect of pH on the Toxicity of Nicotine Injected into the Cockroach <i>Periplaneta americana</i> (L.). CHARLES H. RICHARDSON and L. O. ELLISOR .....   | 305 |
| Nature of <i>Eimeria nieschulzi</i> -growth-promoting Potency of Feeding Stuffs. I. Positive Effect of Gray Shorts. ELERY R. BECKER.....                | 317 |
| Some New and Heretofore Unrecorded Nabidae (Hemiptera). HALBERT M. HARRIS .....   | 323 |

Vol. XIV, No. 4, July, 1940

|   |     |
|---|-----|
| A Functional Method for the Solution of Thin Plate Problems Applied to a Square, Clamped Plate with a Central Point Load.<br>C. J. THORNE and J. V. ATANASOFF ..... | 333 |
| Preliminary Studies on the Use of Dinitro-O-Cresol Dusts in Grasshopper Control, G. C. DECKER AND C. J. DRAKE.....  | 345 |
| Some Reactions of Grasshoppers to Castor Bean Plants.<br>LOUIS A. SPAIN .....   | 353 |
| The Dissimilation of Levulose by Heterofermentative Lactic Acid Bacteria. M. E. NELSON and C. H. WERKMAN .....  | 359 |
| Nutritive Requirements of the Heterofermentative Lactic Acid Bacteria. H. G. WOOD, CHARLES GEIGER and C. H. WERKMAN.....  | 367 |
| The Quantity of Pericarp in Several Hybrid and Inbred Strains of Sweet Corn. W. G. GAESSLER, R. M. HIXON and E. S. HABER .....                                      | 379 |
| The Response of the Plum Grown Under Hillculture Conditions to Modifications in Cultural Treatment. J. M. AIKMAN AND<br>H. E. BREWER .....                          | 385 |
| Motility of the Excised Fore-gut of <i>Periplaneta americana</i> (Orthoptera) in Various Salt Solutions. J. T. GRIFFITHS, JR., and<br>OSCAR E. TAUBER .....         | 393 |
| Effect of Ether on the Toxicity of Certain Fumigants to the Confused Flour Beetle, <i>Tribolium confusum</i> Duval. HAROLD GUNDERSON                                | 405 |
| Index to Volume XIV .....   | 419 |



# CONTENTS

## ABSTRACTS OF DOCTORAL THESES

|  |    |
|--|----|
| Effects of tomato juice on production of flavor contributants in butter cultures. CLEMENT WILFRED ABBOTT .....                                     | 3  |
| Bacteriological studies on swiss-type cheese from pasteurized milk. FRED JOHN BABEL .....  | 6  |
| Organometallic radicals. JAMES CLYDE BAILIE .....  | 8  |
| The effect of decomposition on the lignin of plant materials. JOHN BRUEN BARTLETT .....  | 11 |
| Bacterial dissimilation of citric acid. CARL ROBERT BREWER .....   | 14 |
| Relationship of <i>Achromobacter putrefaciens</i> to the putrid defect of butter. THOMAS JOSEPH CLAYDON .....                                      | 17 |
| Differentiability and continuity properties of solutions of certain partial differential equations of applied mathematics. ARTHUR W. DAVIS .....   | 20 |
| Effects of toxic compounds on the gustatory chemoreceptors in certain diptera. CHRISTIAN CHARLES DEONIER .....                                     | 22 |
| The oxidation of certain polyhydric alcohols by <i>Acetobacter suboxydans</i> . JOHN W. DUNNING .....  | 24 |
| Studies on the fermentative activity of yeast zymin. KENNETH G. DYKSTRA .....  | 27 |
| Effect of treated fats on vitamin A potency. HARRY CHAIM DYME .....  | 29 |
| Effect of growth of microorganisms on acid numbers of fat in cream and butter. EVERETT L. FOUTS .....  | 32 |
| The quantitative spectrographic analysis of soils. RALPH ABIJAH GOODWIN .....  | 35 |
| Use of functionals in obtaining approximate solutions of linear operational equations. GEORGE LLOYD GROSS .....                                    | 37 |
| Effect of ether on the toxicity of certain fumigants to the confused flour beetle, <i>Tribolium confusum</i> Duval. HAROLD GUNDERSON ....          | 39 |
| Relation of the structure of sugars to the chemism of the butyl-acetonic fermentation. JAMES F. GUYMON .....                                       | 40 |
| A classification of the proteolytic micrococci isolated from dairy products. HORACE H. HARNED .....  | 43 |
| The determination of elastic constants by piezo-electric methods. PHILLIP JAMES HART .....   | 46 |
| Radiant heat transfer in ceramic kilns. FRANK B. HODGDON .....   | 48 |
| The accuracy of the plating method for estimating the number of bacteria, actinomyces and fungi in a laboratory sample of soil. NORMAN JAMES ..... | 50 |
| Insecticidal action of some substituted pyrrolidines. JUSTUS G. KIRCHNER .....   | 53 |

Note: Complete copies of these theses may be consulted at the Library, Iowa State College, Ames, Iowa.

|  |     |
|--|-----|
| Relationship between the electron-sharing ability of radicals and the association of organic compounds. FRANC A. LANDEE .....  | 55  |
| Organometallic compounds of group VIII elements. MYRL LICHTENWALTER .....  | 57  |
| The conductance of solutions of organosubstituted ammonium chlorides in liquid hydrogen sulfide. EDGAR ELWYN LINEKEN .....   | 60  |
| Reactions of some high-molecular-weight fatty acid derivatives. MILES R. McCORKLE .....  | 64  |
| Breakdown potentials of gases under alternating voltages. D. OREN McCoy .....  | 67  |
| Ionization constants and insecticidal action of substituted quinolines and tetrahydroquinolines. WILBUR N. OLDHAM .....  | 69  |
| Finite strain analysis in elastic theory. DONALD HILL ROCK .....   | 71  |
| Some studies on swine influenza. I. Comparative study of Hemophilus influenzae suis and Hemophilus influenzae. II. Antibody response to experimental swine influenza. CARLOS T. ROSENBUSCH ..... | 73  |
| Relation between the free energy of soil water and the moisture content of soil. MORELL B. RUSSELL .....   | 76  |
| The relation between methods of saccharification and yield of ethanol from various cereals. LORIN SCHOENE .....  | 78  |
| The culture of fly larvae for use in maggot therapy. SAMUEL WILLIAM SIMMONS .....  | 81  |
| Effect of quartz filters on the distribution of energy in laue patterns. DEAN WALDO STEBBINS .....   | 84  |
| Some factors influencing nitrogen fixation by Azotobacter. JOHN L. SULLIVAN .....  | 87  |
| Some changes produced in growth, reproduction, blood and urine of rats by salts of zinc with certain observations on the effects of cadmium and beryllium salts. WILLIAM R. SUTTON .....         | 89  |
| Derivatives of 1-, 4-, 6-, and 9-substituted dibenzofurans. JACK SWISLOWSKY .....  | 92  |
| Evaluations of consumption in modern thought. ALISON COMISH THORNE .....   | 95  |
| Some anomalous Friedel-Crafts reactions. JOSEPH A. V. TURCK, JR. ....  | 98  |
| Preparation of cholesterolene and various cholestadienes. RALPH LAWRENCE VAN PEURSEM .....   | 101 |
| Differentiation of the vitamins of the B complex and their distribution in certain foods. WALDO RICHARD WYATT .....  | 103 |



# EFFECTS OF TOMATO JUICE ON PRODUCTION OF FLAVOR CONTRIBUTANTS IN BUTTER CULTURES<sup>1</sup>

CLEMENT WILFRED ABBOTT

*From the Department of Dairy Industry, Iowa State College*

Received July 19, 1939

Diacetyl and volatile acids are formed by the citric acid fermenting streptococci when they ferment citric acid in an acid medium and are associated with the aroma of fine butter. In addition to diacetyl, acetylmethylcarbinol which is odorless is always formed in a butter culture and is present in much greater quantities. These two materials are frequently determined together by steam distilling a portion of the culture, to which ferric chloride has been added, into a sodium acetate-hydroxylamine hydrochloride solution. The distillate is heated to form dimethylglyoxime. Nickel chloride is added and a red precipitate of nickel dimethylglyoximate is obtained.

As diacetyl and the associated acetylmethylcarbinol are of primary importance in the production of butter having a high flavor and aroma, attempts were made to increase the yield of acetylmethylcarbinol plus diacetyl by adding various materials to butter cultures containing added citric acid. The intention was not that these materials should serve as substrates but rather that they should increase the amount of acetylmethylcarbinol plus diacetyl formed from the citric acid in the culture.

It was found that certain concentrations of tomato juice produced marked increases in the amounts of acetylmethylcarbinol plus diacetyl produced by butter cultures. In one series of trials, as little as 1 per cent of juice increased the yield by 31.1 per cent, 10 per cent of juice doubled the yield, while the addition of 40 per cent reduced the yield 41.2 per cent below that of cultures containing no juice.

Tomato juices from different sources showed great differences in their effects on the yield of acetylmethylcarbinol plus diacetyl by butter cultures. Some brands were as potent as juices from fresh tomatoes, but none exceeded them. Juices from red and from yellow tomatoes appeared to be equally effective.

No relation could be found between specific gravity, salt content, pH, color after sterilization and the effect of the juice on the yield, but three brands of juice having relatively high salt contents (between 0.8 and 0.9 per cent) were found to depress the yields below those of cultures containing no tomato juice. The suggestion is made that juice intended for use in media be assayed against juice from fresh, ripe tomatoes.

When the acetylmethylcarbinol plus diacetyl content of a culture was determined at frequent intervals, it was found that the initial content was very low, and remained low for several hours, then the amount present increased very rapidly and reached a maximum. After this a gradual reduction in the amounts of these materials occurred. When tomato juice was added to culture, it was found that the rate of production of acetyl-

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<sup>1</sup> Original thesis submitted June, 1939. Doctoral thesis number 521.

methylcarbinol plus diacetyl was increased considerably. This meant that the maximum yield was produced sooner than in those cultures to which no juice was added. The two maxima did not differ significantly, indicating that while tomato juice increased the rate of production of acetylmethylcarbinol plus diacetyl by a butter culture it did not increase the maximum amount formed. After the maxima were past, tomato juice appeared to cause a slightly more rapid decrease in the amount of acetylmethylcarbinol plus diacetyl present in the culture. The increases observed in the first experiments were due to the fact that analyses were made during the period when acetylmethylcarbinol plus diacetyl was being produced more rapidly in the cultures containing tomato juice than in the controls.

Different butter cultures varied considerably in their response to the presence of tomato juice, and the same culture, on two different propagations, showed differences in yield which were practically as great as those observed between the simultaneous inoculations of two cultures into the same milk.

When tomato juice was shaken with calcium carbonate the acidity was reduced considerably but the ability of the juice to increase the yield was not destroyed. When citric acid was added to cultures to provide additional substrate, concentrations of 40 per cent of both neutralized and untreated juices gave lower yields than some other concentrations, but when sodium citrate was added, 40 per cent of untreated juice increased the yield and 40 per cent of neutralized juice produced a much less marked decrease than in the cultures containing citric acid.

Attempts to remove citric acid from tomato juice as the calcium or barium salt were not successful.

The addition of tomato juice did not affect the total acidity produced by a pure culture of *Streptococcus lactis* but lowered the pH below that of a control culture by an amount which remained practically constant throughout the trial.

When tomato juice was added to a pure culture of a citric acid fermenting *Streptococcus* it appeared to alter the conditions of fermentation considerably. The cultures containing tomato juice produced the greatest yield of acetylmethylcarbinol plus diacetyl, which was almost twice that of the control, at a pH of 3.18, while the maximum yield in the control culture occurred at a pH of 4.01. The influence of tomato juice on the yield of acetylmethylcarbinol plus diacetyl by butter cultures seems to result from its action on the citric acid fermenting organisms, and not on *S. lactis*.

Attempts to dry tomato juice by direct evaporation, or by evaporation on filter paper or by evaporating a solution of agar in tomato juice were not successful as the resulting products did not increase the yield. Slow freezing of juice resulted in a very slight concentration of the active materials in the later portions of the juice to freeze. Whole tomatoes were sliced and dried in the sun. It was found that when the dried product was macerated with warm water, the resulting juice was as potent as juice from fresh tomatoes.

Exposure to heat did not appear to destroy the potency of the juice, although sterilization reduced the potency to some extent. When the juice was exposed to air and heated for a long period, the ability to increase the yield was completely destroyed and the juice frequently inhibited production to a greater or lesser extent.



A pulp prepared by forcing tomatoes through a sieve did not increase the yield any more than the clear juice.

Neither ether nor alcohol extracts of dried tomatoes increased the yields more than the residue which remained after extraction.

It appears that the increases produced in the yield are not primarily due to the presence of citric acid in tomato juice and that this substance can at best have only a very slight effect on the yield.

# BACTERIOLOGICAL STUDIES ON SWISS-TYPE CHEESE FROM PASTEURIZED MILK<sup>1</sup>

FRED JOHN BABEL

*From the Department of Dairy Industry, Iowa State College*

Received July 19, 1939

Propionic acid bacteria were found in various cheeses, including Iowa Swiss-type, domestic Swiss, Cheddar and canned Cheddar. Swiss-type cheese having a characteristic sweet flavor generally contained relatively large numbers of propionic acid bacteria, while cheese with a poor flavor generally contained few or no propionic acid bacteria in 0.1 gram of cheese. All the samples of domestic Swiss cheese examined, contained rather large numbers of propionic acid bacteria. About 85 per cent of the samples of Cheddar cheese (of both good and poor quality) contained propionic acid bacteria; there was no correlation between the numbers of these organisms and the quality of the cheese. One sample of canned Cheddar cheese, which had eyes similar to those in Swiss cheese, contained a rather large number of propionic acid bacteria. The results suggest that the fermentation in Swiss-type and domestic Swiss cheese is quite different than in Cheddar cheese.

Eighteen strains of propionic acid organisms (genus *Propionibacterium*) were used in the manufacture of Swiss-type cheese from pasteurized milk for the purpose of determining differences between cultures and with the hope of obtaining a culture that would produce good quality cheese consistently. Several of the strains were rather consistent in the type of flavor produced while others were variable. None of the cultures consistently produced good eye formation.

In most instances, cultures which increased appreciably in number during the ripening period produced cheese having considerable sweet flavor.

Additional trials made with cultures which gave satisfactory results in the original comparisons indicated that certain cultures rather regularly produced cheese having an excellent or good flavor. The culture selected as best produced cheese having an excellent or good flavor in 16 of 17 trials.

A comparison of various amounts of propionic acid culture showed that, by increasing the number of propionic acid bacteria added to the milk, a corresponding increase was obtained in the degree of sweet flavor produced. Cheese receiving an inoculation of 100 ml. of culture per 100 pounds of milk were occasionally criticized as being too sweet. With an inoculation of 25 ml. of culture per 100 pounds of milk, the cheese were considered to have about the desired sweet flavor. The cheese receiving an inoculation of 100 ml. of culture per 100 pounds of milk had a tendency to develop eyes which were a little larger than was desirable, but this was not true in all cases. Cheese made with an inoculation of 5 ml. of culture per 100 pounds of milk gave the best eyes in the majority of instances.

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<sup>1</sup>Original thesis submitted June, 1939. Doctoral thesis number 510.



In several series, the control cheese to which no propionic acid culture was added developed as good a flavor as the cheese made with propionic acid culture. When this was the case, propionic acid bacteria were found in the cheese in large numbers.

Frequently, the four cheese in a series closely resembled each other in eye formation although one cheese was a control while the others were made with propionic acid culture added to the milk. Cheese in which no propionic acid bacteria could be detected in 0.1 gram sometimes developed eyes equal to those in cheese made with added propionic acid culture. These results suggest that organisms in the cheese, other than propionic acid bacteria, can undoubtedly produce eyes in Swiss-type cheese.

When Swiss-type cheese were not salted until 7 days after manufacture, the cheese contained more eyes and the eyes extended nearer the surface than when the cheese were salted in the normal way, the day following manufacture. Delayed salting also tended to produce cheese having more sweet flavor. However, the delayed salting tended to cause the cheese to flatten, which was not the case when the cheese were salted in the normal manner. Since the cheese used in these experiments were about 15 pounds in weight, there probably was less flattening than if a heavier cheese had been made. A 2-day delayed salting period slightly favored the production of more desirable eyes and the sweet flavor was somewhat more conspicuous; the cheese also retained their original shapes better.

Excessive swelling, due to the production of many large eyes, could be stopped by placing the cheese in a cold room at about  $3.3^{\circ}$  to  $4.4^{\circ}$  C. Swiss-type cheese held at about  $21.1^{\circ}$  to  $22.2^{\circ}$  C. showed first signs of swelling in 3 or 4 days while with holding at  $10.0^{\circ}$  to  $12.7^{\circ}$  C. the first signs of swelling were evident in 3 or 4 weeks. Cheese held for a time at the higher temperature tended to have eyes which were not quite as smooth as the eyes in the cheese held at the lower curing temperature.

The addition of propionic acid bacteria to pasteurized milk made into cheese favored the production of a higher volatile acidity and the cheese-fat contained more free acids than cheese made from milk to which no propionic acid bacteria were added.

The pH determinations on Swiss-type cheese aged 3 months indicated there was no definite correlation between pH and quality. There was very little difference between the pH values of all the cheese examined. Although these data are not in agreement with the results of other investigators, it should be noted that only a small amount of acid is developed in Swiss-type cheese in comparison to that developed in regular Swiss cheese made in the kettle. The lactose concentration in Swiss-type cheese is also very low and therefore does not favor extensive lactic acid fermentation.

Two series of cheese contained large numbers of *Escherichia-Aerobacter* organisms; they were more objectionable from the standpoint of eye formation than flavor since the eyes were small and numerous. One series of cheese contained large numbers of anaerobic spore-forming rods (genus *Clostridium*); they were more objectionable from the standpoint of flavor than eye formation.

# ORGANOMETALLIC RADICALS<sup>1</sup>

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*From the Department of Chemistry, Iowa State College*

Received July 19, 1939

The work of Krause and collaborators<sup>2</sup> first showed that triaryllead compounds were readily obtained from the reaction of lead chloride and arylmagnesium halides. These compounds were shown to dissociate in solution, and they possessed other properties remindful, to some extent, of the triarylmethyl radicals.

In the present investigation the following triaryllead compounds are reported for the first time: tri-*p*-methoxyphenyllead (m. p. 198-200° dec.), tri-*o*-methoxyphenyllead (m. p. 201° dec.), tri-*p*-ethoxyphenyllead (m. p. 178-179° dec.), tri-*o*-ethoxyphenyllead (m. p. 170-171° dec.), tri- $\alpha$ -naphthyllead (m. p. 268-269° dec.), trimesityllead (m. p. above 325°), and tri-*m*-tolyllead (m. p. 109°).

The thermal decomposition of trivalent lead compounds causes deposition of metallic lead and formation of the stable tetravalent lead compound.



The decomposition of a number of trivalent lead compounds has been carried out in xylene solution and the effect of the radical attached to lead upon the ease of decomposition has been determined. The following series of radicals has been obtained, arranged in the order of the decreasing stability of the trivalent lead compounds:

Mesityl, cyclohexyl,  $\alpha$ -naphthyl > *o*-ethoxyphenyl,  
*o*-methoxyphenyl, *o*-tolyl > *p*-ethoxyphenyl, *p*-  
methoxyphenyl, *p*-tolyl > *m*-tolyl, phenyl > ethyl,  
methyl.

The reaction of triphenylmethyl with the binary system (Mg + MgI<sub>2</sub>) leads to the formation of triphenylmethylmagnesium iodide<sup>3</sup>. Treatment of trivalent lead compounds with the binary mixture caused reaction to occur, but stable compounds of the type R<sub>3</sub>PbMgI were not obtained. With triphenyllead, tri-*p*-tolyllead, tri-*p*-methoxyphenyllead, tri-*p*-ethoxyphenyllead, and triethyllead, the products obtained were an organomagnesium compound, the tetravalent lead compound, and free lead. With magnesium iodide in the absence of free magnesium, triphenyllead and tri-*p*-tolyllead gave the respective triaryllead iodides. The mechanism proposed for the reaction of trivalent lead compounds with the binary system involves the primary reaction with magnesium iodide to produce the triaryl- (or alkyl) lead iodide, which reacts with free magnesium to form an unstable compound of the type R<sub>3</sub>PbMgI. The latter compound

<sup>1</sup>Original thesis submitted July, 1938. Doctoral thesis number 482.

<sup>2</sup>Krause and collaborators, *Ber.*, 52:2165 (1919); 54:2060 (1921); 55:888 (1922).

<sup>3</sup>Gomberg and Bachman, *J. Am. Chem. Soc.*, 49:236 (1927); 52:2455 (1930). Gilman and Fothergill, *ibid.*, 51:3149 (1929).



decomposes to  $\text{RMgI}$  and  $\text{R}_2\text{Pb}$ , and the unstable divalent lead compound further decomposes to  $\text{R}_4\text{Pb}$  and lead.

The highly stable *ortho* substituted triaryllead compounds did not react with the binary system to produce a Grignard reagent, but the triaryllead iodides were formed. In this manner were obtained tri-*o*-methoxyphenyllead iodide (m. p. 122-123°), trimesityllead iodide (m. p. 200-201°), and tricyclohexyllead iodide. Tetraphenyllead and tetra-*p*-methoxyphenyllead failed to react with the binary system.

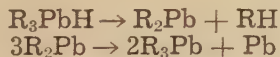
From the reaction of tri-*p*-methoxyphenyllead and iodine in chloroform solution there was obtained tetra-*p*-methoxyphenyllead, di-*p*-methoxyphenyllead diiodide (m. p. 122-123°), and lead iodide. The same reaction with tri-*o*-methoxyphenyllead gave tri-*o*-methoxyphenyllead iodide (m. p. 122-123°), and lead iodide, while tri-*p*-ethoxyphenyllead gave tri-*p*-ethoxyphenyllead iodide (m. p. 99-100°) and lead iodide. Treatment of triphenyllead in chloroform with dry hydrogen chloride caused cleavage to diphenyllead dichloride and lead chloride.

The reactions of several trivalent lead compounds and organolead halides with sodium in liquid ammonia have been carried out. Treatment of a triaryl- (or alkyl) lead halide with one equivalent of sodium in liquid ammonia causes removal of sodium halide and the triaryl- (or alkyl) lead compound is formed. Further addition of another equivalent of sodium causes the formation of the  $\text{R}_3\text{PbNa}$  compound. The same result may be attained by treating the  $\text{R}_3\text{Pb}$  compound with one equivalent of sodium. If the sodium compound is treated with a compound containing a reactive halogen atom, such as benzyl chloride, the unsymmetrical tetravalent lead compound results.



In this manner have been prepared triphenylbenzyllead, tri-*p*-tolylbenzyllead (m. p. 81-82°), tri-*p*-ethoxyphenylbenzyllead (m. p. 76-77°), tri-*o*-methoxyphenylbenzyllead (m. p. 80-81°), and tricyclohexylbenzyllead (dec. 228°). From sodium triphenyllead and benzohydril chloride was obtained triphenylbenzohydrillead (m. p. 122°).

Attempts were made to prepare triaryl- and trialkyl- lead hydrides by ammonolysis of the corresponding sodium compound with ammonium halide in liquid ammonia solution. The products isolated and the color changes observed indicated that the hydrides were formed initially and decomposed as follows:



The reaction between triphenyllead chloride, bromide, or iodide and triphenylmethylmagnesium chloride resulted in the formation of a lemon-yellow, crystalline compound (m. p. 196-197° dec.), which is probably triphenyltriphenylmethyllead. Molecular weight determinations made by measuring the freezing point depression of dilute benzene solutions gave values which indicated that appreciable dissociation occurred within the molecule. In benzene solution oxidation took place slowly with the formation of triphenylmethylperoxide and triphenyllead, while thermal decomposition in xylene solution caused the deposition of free lead and formation of tetraphenyllead. The reactions with hydrogen chloride and iodine were inconclusive, but demonstrated the sensitivity of the compound toward those reagents. With dry hydrogen chloride in chloroform solution, the products were lead chloride and triphenylcarbinol, and in

petroleum ether (b. p. 60-66°) diphenyllead dichloride was obtained. Iodine in chloroform solution caused immediate precipitation of lead iodide and the only other product isolated in the pure state was a trace of triphenyllead iodide.

The reaction with sodium in liquid ammonia resulted in the formation of the sodium derivatives of triphenylmethyl and triphenyllead, which, on ammonolysis with ammonium bromide, gave triphenylmethane and triphenyllead. When benzyl chloride was used in place of ammonium bromide, the products were 1, 1, 1, 2-tetraphenylethane and triphenylbenzyllead.

The observations made on the compound indicate that the bond between the tertiary carbon atom and the lead atom is very weak, and possibly dissociation to triphenylmethyl and triphenyllead occurs. However, attempts to prepare the compound by mixing equimolecular quantities of triphenylmethyl and triphenyllead have met so far with no success.

The corresponding compound of tin, triphenyltriphenylmethyln, was prepared from triphenyltin chloride and triphenylmethylmagnesium chloride. It is a colorless, crystalline compound, m. p. 272-273° dec., and molecular weight measurements made cryoscopically in benzene gave values which indicated that dissociation did not occur. The reaction with sodium in liquid ammonia with subsequent ammonolysis gave triphenylmethane and triphenyltin. The greater stability of the carbon-tin bond over the carbon-lead bond was demonstrated by the comparatively slow cleavage of triphenyltriphenylmethyln with hydrogen chloride, in which a phenyl group was removed and diphenyltriphenylmethyln chloride (m. p. 210°) was formed.

The reaction between tri-*o*-methoxyphenyllead iodide and triphenylmethylmagnesium chloride resulted in the formation of the yellow crystalline tri-*o*-methoxyphenyltriphenylmethyllead (m. p. 145-146°).

When a benzene-ether solution of triphenylmethylmagnesium chloride was treated with lead chloride, free lead was deposited, and triphenylmethyl (identified as the peroxide) was formed.

It has been found that the addition of one equivalent of iodine to a chloroform solution of tetraphenyllead gives excellent yields of triphenyllead iodide.

Tri-*m*-tolyllead bromide (m. p. 146-147°) was prepared by the reaction of bromine on tetra-*m*-tolyllead in pyridine solution at low temperatures.

The following new tetraaryllead compounds have been prepared by thermolysis of the corresponding triaryl compounds: tetra-*m*-tolyllead (m. p. 122-123°); tetra-*p*-methoxyphenyllead (m. p. 145-146°); tetra-*p*-ethoxyphenyllead (m. p. 110°); tetra-*o*-methoxyphenyllead (m. p. 148-149°); tetra-*o*-ethoxyphenyllead (m. p. 219-220°).



# THE EFFECT OF DECOMPOSITION OF THE LIGNIN OF PLANT MATERIALS<sup>1</sup>

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Received July 19, 1939

The decomposition of organic matter is a complex process and presents innumerable problems. One of the most perplexing of these is the question of lignin decomposition. The fact that lignin decomposes so much more slowly than the other constituents of plant materials means that there is a relative accumulation of lignin in the soil with the result that this complex usually forms from 30 to 40 per cent of soil organic matter. This led some of the earlier workers to the belief that lignin does not decompose. More recent work has definitely shown that this assumption was false. In fact it is obvious that if lignin did not decompose it would soon accumulate in soils to such an extent as to be readily recognizable by chemical means.

One of the major difficulties in studying lignin decomposition is the fact that its constitution is unknown. The various workers are not even in general agreement as to either its constituent groupings or the degree of complexity of the molecule.

The fact that the properties of organic materials undergo marked changes upon decomposition in the soil, coupled with the fact that lignin is such an important constituent of the decomposed residues suggests that the properties and chemical structure of lignin may also change appreciably during decomposition. If changes in the lignin of plant material do occur as rotting proceeds it may well be that they will account for an appreciable portion of the changes in the properties of the total material. The general objective of this investigation was to determine the nature and extent of some of the changes which decomposition may bring about in lignin and also whether such changes as may be measured actually produce corresponding changes in the rotted residues.

Although it is well known that no method of lignin isolation will give a product identical with the original lignin in the plant tissue, the assumption was made in this work that any changes measured in lignin isolated by the same method from all the materials studied would give a relative measure of changes in the lignin of the original plant tissue. Since it was known that the nitrogenous material synthesized by microorganisms during decomposition considerably affects the purity of lignin prepared from rotted residues by the majority of the methods of isolation, the first problem encountered was the choice of a procedure in which this disturbance could be minimized. In the case of the strong mineral acid methods dilute acid pretreatment has been found to considerably reduce the protein content of most materials and thereby eliminate to some extent the nitrogenous impurities of the lignins prepared by these methods. In the case of the alkaline extraction methods the protein disturbance is

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<sup>1</sup> Original thesis submitted July, 1938. Doctoral thesis number 479.

even more difficult to eliminate, so that none of this group of methods was considered suitable for these studies. Many hydroxy compounds, such as alcohols, glycols, phenols, etc., in the presence of a catalyst, form soluble derivatives of lignin, but even on prolonged treatment or repeated treatments the yields obtained by most of these methods of preparation are poor. Certain organic acids have recently been shown to condense with lignin to give good yields of soluble products. In this work the lignin prepared by one of these, the thioglycollic acid method, was compared with the lignin obtained from the same materials by the 72 per cent sulphuric acid method. It was found that the nitrogen content of the thioglycollic preparations was not sufficiently lower than that of the sulphuric acid lignins to justify the adoption of this procedure. In view of the fact that more is known of the nature and extent of the errors involved in the mineral acid methods and the means which may be employed in overcoming them, one of these methods, the sulphuric acid procedure, was finally adopted in this work. This method was chosen in preference to the 43 per cent hydrochloric acid method because the latter offers no known advantage and is much less pleasant to use.

In this work cornstalks, rye straw, oat straw, and wheat straw were decomposed aerobically for periods of three and six months. Sulphuric acid lignin was isolated from both the rotted residues and the original materials. The base exchange capacities of the plant materials and the lignin preparations were determined by an ammonium acetate distillation method and by a barium acetate titration method. Since the former of these two methods gave much lower values, at least partially attributable to the solution effect of the ammonium acetate, it was concluded that the barium method gave a more reliable measurement of the replacement capacity. The change in the sorption capacity of the plant materials with decomposition, calculated upon the basis of unit weight of original material, and the contribution of the lignin to the total exchange capacity of the residues and the original materials were determined. The role played by the ash content of the lignin in the base sorption capacity was investigated. Finally the methoxyl content of the lignin preparations and their reactivity to alkaline oxidation with sodium hypoiodite were measured. The results obtained in these studies may be summarized as follows:

1. Approximately one-third of the lignin in the four plant materials and about two-thirds of the materials themselves were removed in six months.

2. The exchange capacity of the ash-free lignins from the rotted residues was three or four times as great as that of those from the original materials, but the sorption capacities of the residues and the parent materials did not differ greatly, when both were calculated on the basis of unit weight of original material.

3. A major share of the replacement capacity of the rotted residues was due to their lignin content, but in the parent materials only a small share of the sorption capacity could be attributed to lignin.

4. The sorption activity of the ash content of the lignin preparations was found to be negligible.

5. The fact that the greatly increased sorption capacity of the lignin with decomposition was not accompanied by much change in the replacement capacity of the plant materials can in all probability be attributed

to a simultaneous removal of non-lignious material possessing base exchange properties.

6. A loss of from 27 to 40 per cent of the methoxyl groups of the isolated lignins occurred during six months' decomposition.

7. The reactivity of lignin to alkaline oxidation with sodium hypiodite was not much changed by decomposition.



# BACTERIAL DISSIMILATION OF CITRIC ACID<sup>1</sup>

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Received July 19, 1939

The dissimilation of citric acid by bacteria of the coli-aerogenes group and citrate-fermenting streptococci as influenced by environmental factors was investigated. The ability of these organisms to attack citric acid has been noted by many workers but no adequate physiological study of their metabolic activities on citric acid has appeared.

The two groups of bacteria were found to attack citric acid in distinctly unlike manner. Generic differences were noted among the citrate-fermenting members of the coli-aerogenes group.

It was found that all of the bacteria studied dissimilate citric acid by reactions which are unlike those postulated for the oxidation of citric acid by animal cells (Martius, 1938; Krebs and Johnson, 1937).

The dissimilation of citric acid by coli-aerogenes bacteria is essentially a degradation to simpler organic acids. The products formed in greatest abundance are acetic, formic and succinic acids and carbon dioxide. In the presence of oxygen, the acidic products of the fermentation of citrate are oxidized mostly to carbon dioxide and water with a simultaneous assimilation of a part of the substrates to complex, carbohydrate-like materials.

The two citrate-fermenting genera of the coli-aerogenes group, *Aerobacter* and *Citrobacter* are unlike in their attack on citrate. *Aerobacter* forms larger quantities of acetic acid and carbon dioxide and smaller quantities of succinic acid than *Citrobacter*. *Aerobacter* also produces small amounts of ethyl alcohol, acetylmethylcarbinol, 2,3-butylene glycol and lactic acid from citrate, none of which has been found in the fermentation of citric acid by *Citrobacter*. Varying quantities of formic acid were formed from citrate by *Aerobacter* but no formate was found in the fermentations of citrate by *Citrobacter*. Approximately one-half of the carbon of citric acid is converted to succinic acid by *Citrobacter*.

No conclusive evidence for the accumulation and subsequent decomposition of any metabolite was obtained. The products of citrate fermentation by *Aerobacter* are formed in the same ratio throughout the process. Some evidence with *Citrobacter* that the ratio of succinic acid to acetic acid decreases with time was obtained. The decrease was not great.

*Aerobacter indologenes* ferments citric, oxaloacetic, l-malic, fumaric, pyruvic and formic acids but not aconitic, citraconic, itaconic,  $\alpha$ -OH-isobutyric, tricarballic, succinic and acetic acids. Under aerobic conditions the fermentable acids are oxidized. Succinic and acetic acids are also dissimilated aerobically. Aconitic acid is slowly oxidized after an induction period during which it may add water enzymically to form citric acid.

The oxidation of citric acid by coli-aerogenes bacteria follows the

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<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 538.

normal anaerobic dissimilation of citrate, which may take place in an intensely aerated medium. Hydrogen gas is produced by *Aerobacter indologenes* during the oxidation of citrate and that of succinate or acetate. *Citrobacter* did not form hydrogen aerobically but did oxidize citric acid more rapidly and completely than did *Aerobacter*. The assimilation of carbohydrate-like compounds during citrate oxidation by coli-aerogenes bacteria is inhibited by appropriate concentration of sodium azide (Clifton and Logan, 1939).

The anaerobic dissimilation of citric acid by *Aerobacter indologenes* was completely inhibited by low concentrations of arsenite, bisulfite and monoiodoacetate. Sodium fluoride partially inhibited the fermentation. Malonate stimulated gas production from citrate and, to an even greater degree, from malate and fumarate. Arsenite and monoiodoacetate totally inhibited aerobic citrate dissimilation but fluoride and pyrophosphate had only a slight effect. The respiration was partly inhibited by cyanide.

Evidence for the conversion of citric acid through oxaloacetic and pyruvic acids was presented. The two keto acids are rapidly dissimilated to the normal products of citrate decomposition by coli-aerogenes bacteria. Neither of the postulated acids was isolated and identified.

The differences in the products formed by *Aerobacter indologenes* from citrate and from glucose may be explained by two facts. First, the relative states of oxidation of the substrates are unlike. Secondly, (and probably more important) the enzymic action on the proposed intermediate compounds of *Aerobacter* grown in the two substrates shows that the relative concentrations of the enzymes concerned are determined by the natures of the growth substrates. *Aerobacter* grown in glucose converts pyruvate to relatively large quantities of 2,3-butylene glycol and small quantities of succinic acid (similar to a normal fermentation of glucose. Cells grown in citrate convert pyruvate to relatively large quantities of succinic acid and small quantities of glycol (similar to the fermentation of citrate).

It is postulated that coli-aerogenes bacteria ferment citric acid by first cleaving it to acetic and oxaloacetic acids. The latter may form succinic acid upon reduction or may be decarboxylated to pyruvic acid. Pyruvate has been shown by previous investigators to give rise to the remainder of the products found.

This scheme of dissimilation requires modification to fit the experimental facts obtained with *Citrobacter*. To account for the large yields of succinic acid, it is necessary to postulate that a part of this acid is formed by the condensation of acetic acid. No conclusive evidence for such a reaction in bacterial physiology has yet been published.

*Streptococcus paracitrovorus* does not readily dissimilate citric acid in the absence of sugar but does attack citric acid relatively vigorously in the presence of small quantities of glucose or lactose. The effect of glucose and lactose in initiating the dissimilation of citric acid is catalytic.

The sugars which act catalytically are themselves fermented to approximately equimolar quantities of carbon dioxide, ethyl alcohol and lactic acid. The dissimilation of a combined substrate of citrate and glucose forms, in addition, acetic acid, acetylmethylcarbinol, 2,3-butylene glycol and under certain conditions, pyruvic acid which acts as an intermediate compound. The fermentation of a relatively small quantity of

glucose in a combined substrate may permit the decomposition of a several-fold quantity of citric acid.

Pyruvate is dissimilated by *S. paracitrovorus* to products similar to those formed from a combined substrate of citrate and glucose.

The dissimilation of citric acid and glucose by the citrate-fermenting streptococci is essentially an anaerobic process; little oxygen is consumed in aerobic experiments. Arsenite, which inhibits the dissimilation of citric acid by coli-aerogenes bacteria and animal cells, has no effect on the fermentation of citrate by *S. paracitrovorus*.

The addition of several common biological hydrogen donors and acceptors to suspensions of *S. paracitrovorus* in citrate, failed to stimulate the dissimilation of citric acid in a manner comparable to the action of glucose or lactose. It thus appears that the catalytic activity of these sugars in the fermentation of citrate is not entirely due to the transfer of hydrogen.

Since milk contains lactose, it is probable that the fermentation of the citric acid of milk is catalyzed in a manner similar to that demonstrated. The study of the metabolism of butter culture bacteria should thus include the sugar of milk as well as citric acid.

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# RELATIONSHIP OF *ACHROMOBACTER PUTREFACIENS* TO THE PUTRID DEFECT OF BUTTER<sup>1</sup>

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Received July 19, 1939

The putrid type of cheesiness in butter is a common defect that is encountered in the main butter producing countries. It is characterized by a marked odor suggestive of limburger cheese. While opinions differ regarding the cause of the defect, it is generally accepted as being of bacterial origin. Derby and Hammer (2) isolated an organism from putrid butter which they named *Achromobacter putrefaciens*. It was capable of producing a typical putrid defect in butter. Cullity and Griffin (1) reported that *Ach. putrefaciens* had been isolated from putrid butter and from water supplies of some butter plants in Australia. Recent work in western Canada (4) indicates that the organism has been obtained from surface taint butter and from creamery water supplies.

Commercial putrid butter was plated on beef infusion agar. Colonies obtained from the plates were cultured in litmus milk, inoculated into thoroughly pasteurized cream and the cream churned. Samples of the resulting butter were held at 21° and 5° C. and examined frequently for the development of the putrid defect. The results indicated that the predominating organisms on beef infusion agar plates poured with the commercial butter were not responsible for the defect.

Since it has been shown by Long and Hammer (3) that the Burri smear technic demonstrates types of organisms in butter that are not made evident by the usual plating procedure, a modification of the method was used in examining the defective samples. Plates were poured with beef infusion agar containing skim milk and fat emulsion. They were allowed to solidify, marked into sectors and smeared with tiny portions (about 1/20,000 gram) of the defective butter. During incubation of the smeared plates examinations were made frequently for the appearance of various types of colonies. By means of the technic and certain enrichment procedures, *Ach. putrefaciens* was obtained from various samples of putrid butter. With few exceptions it was the only organism obtained that was capable of reproducing the typical defect. It was nearly always obtained in small numbers.

The isolation of *Ach. putrefaciens* was facilitated by inoculating the original butter into thoroughly pasteurized cream, churning the cream and smearing portions of the resulting butter on agar after the defect had developed. Inoculation of defective experimental butter into cream for the production of second or third generation samples occasionally resulted in the isolation of the organism when it had not been obtained previously. Incubation of the inoculated cream, the experimental butter and the smeared plates at 5° to 10° C., rather than at 21° C., further facilitated the isolation.

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<sup>1</sup> Original thesis submitted June, 1939. Doctoral thesis number 512.

Of 58 commercial butter samples having the typical putrid defect, 41 (70.7 per cent) yielded *Ach. putrefaciens* and 6 (10.3 per cent) yielded an apparent variant of the organism. Three (5.2 per cent) yielded other types of organisms capable of producing objectionable odors in butter, which were not typical of the putrid defect. From 8 (13.8 per cent), the causative organism could not be isolated.

The age of the butter seemed to bear a relationship to the ease with which *Ach. putrefaciens* was isolated since fresh samples yielded the organism more readily than older samples. Possibly, this was due to the fact that in the older samples the organism had died before the examination was begun. In experimental butter *Ach. putrefaciens* decreased in numbers as the butter aged and became increasingly difficult to regain. The fact that *Ach. putrefaciens*, although growing abundantly when once established, does not readily initiate growth on artificial media may also account for the failure to isolate the organism more consistently from putrid butter.

*Ach. putrefaciens* was isolated from the water supply of a plant having difficulty with the putrid defect. Its presence in water indicates one of the probable sources of the organism and is in agreement with the observations of other workers. Examinations of a number of churns in plants not encountering the defect failed to yield the organism. While this may indicate that *Ach. putrefaciens* is not a common type under normal conditions, the difficulties associated with the isolation of the organism must be considered in interpreting the results.

When inoculated into pasteurized cream used for churning, *Ach. putrefaciens* produced a typical putrid defect in unsalted butter at 21° and 5° C. The defect developed when the organism was added to the cream in such small amounts that recovery was difficult. The defect was also produced when *Ach. putrefaciens* was added to the water used to wash the butter in such small amounts that it was regained with difficulty. In each case the defect passed through the same stages that occur in commercial butter showing the typical defect.

The putrid defect was observed in samples of commercial putrid butter with pH values varying from 5.8 to 6.8 which indicates that the defect is not necessarily associated with over-neutralization of the cream. *Ach. putrefaciens* was capable of producing the putrid defect in unsalted butter made from lots of cream adjusted to pH values varying from 5.2 to 7.8; no defect developed when the pH of the cream was 4.2.

While salt tended to inhibit the development of the defect produced by *Ach. putrefaciens*, it was not entirely effective unless the butter was thoroughly worked. Five per cent butter culture added to the cream had an inhibitory effect on the production of the putrid defect in unsalted butter. However, the use of butter culture is not a completely effective control measure under all conditions since some of the commercial putrid butter was made from cream containing butter culture.

Because of its characteristics, its action in experimental butter, and its presence in various lots of commercial putrid butter, *Ach. putrefaciens* is considered an important cause of the putrid type of cheesiness in butter.

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# DIFFERENTIABILITY AND CONTINUITY PROPERTIES OF SOLUTIONS OF CERTAIN PARTIAL DIFFERENTIAL EQUATIONS OF APPLIED MATHEMATICS<sup>1</sup>

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Received July 19, 1939

In 1914 Erhard Schmidt<sup>2</sup> published a paper in which, with the aid of certain simple hypotheses, he obtained by elementary methods the breaks in the Newtonian potentials. The purpose of this investigation is to use the methods developed by Schmidt to study the continuity properties and to obtain the breaks in the derivatives of the volume and surface integrals of a generalized potential function  $H/r$ .

The generalized potential function is defined to be a particular solution of the homogeneous linear partial differential equation of the second order.  $H$  is a function of  $x_1 - \xi_1, x_2 - \xi_2, x_3 - \xi_3$ , where  $x_1, x_2, x_3$  are the variables of integration and  $\xi_1, \xi_2, \xi_3$  are the coordinates of the point  $P$  at which the potential is desired;  $r$  is the distance from  $P$  to the element of integration. This form of  $H$  necessarily restricts the differential equation to one with constant coefficients.

With the aid of Stokes' theorem and generalized Green's theorems for three dimensions, it is possible to obtain three equations involving the integrals defining the generalized potential of a volume, simple surface and double surface distribution. The continuity properties and the breaks in the derivatives of the generalized potentials are obtained from these three equations.

The assumption is made that a piece  $b$ , in the neighborhood of the point  $P$ , may be cut from the surface such that by proper choice of a rectangular coordinate system this piece may be represented by an equation of the form  $x_3 = F(x_1, x_2)$ . With this condition the following three theorems are proven by mathematical induction.

Theorem I. If  $F$  and  $H$  are of class  $C^k$  and the density is of class  $C^{k-1}$  then the generalized potential of a volume distribution and its partial derivatives up to and including the  $(k)$ th order are continuous both inside and outside of the body and have continuous limiting values on both sides of the surface.

Theorem II. If  $F$  and  $H$  are of class  $C^{k+1}$  and the surface distribution is of class  $C^k$  then the generalized potential of a simple surface distribution and its partial derivatives up to and including the  $(k)$ th order have continuous limiting values on both sides of the surface.

Theorem III. If  $H$  is of class  $C^{k+2}$  and  $F$  and the surface distribution are of class  $C^{k+1}$  then the generalized potential of a double surface distribution and its partial derivatives up to and including the  $(k)$ th order have continuous limiting values on both sides of the surface.

<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 531.

<sup>2</sup> Schmidt, Erhard. *Bemerkung zur potentialtheorie*. Mathematische Abhandlungen. H. A. Schwarz gewidmet. p. 365-383. Julius Springer, Berlin. 1914.

When the functions contained in the generalized potential integrals satisfy certain differentiability and continuity conditions, it is shown that the breaks in the  $(n + 1)$ st order derivatives of these generalized potentials are given by recursion. For example, the breaks in the  $(n + 1)$ st order derivatives of the generalized volume potential are obtained from the breaks in the  $(n)$ th order derivatives of the generalized potential of a volume and a simple surface distribution. Similar relationships are shown to exist for the breaks in the  $(n + 1)$ st order derivatives of the generalized potentials of a simple surface and a double surface distribution.

The theory has been applied to the following two partial differential equations and the breaks in the generalized potentials and their first and second order derivatives have been found.

$$\begin{aligned}\Delta u + k^2 u &= 0, \\ \Delta u - 2 \left( \frac{\partial u}{\partial x_1} + \frac{\partial u}{\partial x_2} + \frac{\partial u}{\partial x_3} \right) + 3u &= 0.\end{aligned}$$

# EFFECTS OF TOXIC COMPOUNDS ON THE GUSTATORY CHEMORECEPTORS IN CERTAIN DIPTERA<sup>1</sup>

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Received July 19, 1939

A study is made of the gustatory chemoreceptors in certain Diptera. The tarsal chemoreceptors of houseflies, *Musca domestica* L., were tested with 1 molar sucrose solutions. The solutions were offered the flies: 1) with a brush, both with and without the added mechanical stimulus of a needle; 2) in a small glass dish, where a screen wire served as a mechanical stimulus. Both water-satiated and unsatiated flies were tested. Responses of an olfactory nature were not found. Responses of a gustatory nature were initiated when the chemoreceptors of the tarsi came in contact with the solutions. Houseflies were found to be sensitive to very small amounts of sucrose solution.

It was found that these tarsal chemoreceptors enable the housefly to select discriminately the substances that will be touched by the proboscis. An experimental procedure is presented for determining the repellent effects of toxic compounds on the gustatory chemoreceptors. Positive proboscis responses were stimulated when the chemoreceptors on the tarsi came in contact with a sucrose solution. The repellence of various toxic compounds was determined by a comparison of the responses, initiated by the experimental solutions, with the normal positive responses initiated by a 1 molar sucrose solution.

In the housefly, the chemoreceptors of the proboscis were found to be more sensitive to certain compounds than were the chemoreceptors on the tarsi. A solution which was not repellent to the tarsal chemoreceptors might be definitely repellent to the chemoreceptors on the proboscis. Compounds that were repellent to the proboscis caused the flies to instantly withdraw their mouth parts from a solution, even though the tarsal chemoreceptors had initiated a positive response. Whether a fly would or would not ingest a solution was also determined.

Concentrations of the following compounds (g./100 cc.) in 1 molar sucrose solutions were not repellent, and did not materially inhibit feeding: sodium fluoride at 3.5 grams, sodium fluosilicate at 0.5 gram, arsenious acid at 1.84 grams, potassium fluoride at 10.0 grams, potassium iodate at 10.0 grams, and sodium iodate at 9.0 grams.

Definite repellence to the tarsal chemoreceptors was shown by the remaining compounds tested at and above the following concentrations (g./100 cc.) in 1 molar sucrose: mercuric chloride at 0.5 grams, arsenic acid at 0.25 gram, barium chloride at 2.5 grams, potassium salicylate at about 10.0 grams, ammonium iodide at 5.0 grams, sodium iodide at 7.5 grams, and ammonium iodate at 2.0 grams.

Strong concentrations of mercuric chloride had a definite inhibitory effect on the chemoreceptors of the tarsi. The flies gradually recovered their normal response after 1 to 5 hours.

<sup>1</sup>Original thesis submitted March, 1939. Doctoral thesis number 505.



*Cochliomyia americana* C. & P. and *Phormia regina* Meigen were also shown to have on their tarsi and mouth parts chemoreceptors which function as gustatory organs. Both species gave high positive responses to sucrose solutions.

*C. americana* were found to be repelled more strongly by 1.0 per cent mercuric chloride in 0.5 molar sucrose than by the same concentration of poison in 1.0 molar sucrose solution. *P. regina* were repelled less by mercuric chloride at 48 hours of age than they were at 24 hours of age. The flies 48 hours of age were not repelled by a 0.25 per cent concentration of this poison, but the feeding responses were slightly lower. Sex difference was not an important variable in the blowfly populations studied.

*C. americana* and *P. regina* in cage tests were found to distinguish, by contact with the tarsi, the presence of a toxic compound in sucrose solution offered as food. The flies were found to die of starvation when 2.0 per cent mercuric chloride was added to the sucrose offered. The results were comparable to those obtained for the responses of mounted flies. The death rate was greater, and the effects of starvation more rapid in cage tests conducted during the summer months.

# THE OXIDATION OF CERTAIN POLYHYDRIC ALCOHOLS BY *ACETOBACTER SUBOXYDANS*<sup>1</sup>

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Received July 19, 1939

Even in the presence of the astounding advances in the field of organic synthesis which have been made in the past decades, there still remain compounds more readily prepared by the transformations brought about by microbiological catalysts. When it is desired to carry out such a chemical transformation by the use of bacterial enzymes, three factors are of prime importance. In the first place, the proper substrate compound must be selected; secondly, the appropriate organism must be found; and thirdly, the optimum conditions under which the best yields are obtained must be determined.

The experiments of this thesis, developed with the above factors in mind, were inaugurated because of the interest of *i*-inositol in regards to yeast growth stimulants. However, the peculiarities of the fermentation of *i*-inositol by *Acetobacter suboxydans* seemed to warrant more detailed study. It was also seen that, if a suitable means were found for the oxidation of *i*-inositol by *Acetobacter suboxydans*, fermentations of related polyhydric-cyclohexanols could possibly be carried out.

## METHODS

The culture of *Acetobacter suboxydans* used in the experiments for this thesis was obtained from the American Type Culture Collection and is listed as No. 621. The stock cultures were carried on sorbitol-yeast extract agar slants. This stock culture was transferred and subcultured in two different media, one consisting of 10 per cent sorbitol and 0.5 per cent yeast extract and the other of 3 per cent inositol, 0.05 per cent sorbitol, and 0.5 per cent yeast extract.

The course of the conversion of the *i*-inositol into the reducing fermentation product was followed by use of the Shaffer-Hartmann method. During the preliminary experiments, since the glucose factor for the reducing compound was not known, the factor for sorbose (1.25) was used in order that comparative values for the fermentation compound could be calculated. After the fermentation compound had been separated and recrystallized several times, varying quantities were titrated by the Shaffer-Hartmann method to obtain the glucose factor. The factor obtained was 3.18. The use of this figure, however, gave percentage conversion values which were too high. That is, the amount of fermentation compound produced, calculated by the use of this factor, equalled 120 per cent of the amount of inositol initially in the medium. Since this glucose factor is too high and since a satisfactory explanation has not yet been deduced, all the data concerning yields of the inositol fermentation product have been calculated as sorbose.

<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 501.

## EXPERIMENTAL

*A. Investigation of the Growth Factor in the Fermentation of i-inositol*

By subculturing *A. suboxydans* on an inositol-yeast extract medium, it was found that the medium would not support growth of the organism. The presence of a small amount of sorbitol in the medium, however, as supplementary substrate made possible the oxidation of the inositol by the organism.

Varying amounts of sorbitol were added to 3 per cent inositol-0.5 per cent yeast extract medium. These media were sterilized, inoculated with centrifuged and washed *A. suboxydans* cells, and incubated at 30° C. It was found that the optimum concentration of sorbitol in the inositol-yeast extract medium for oxidation of the inositol was 0.1 to 0.05 per cent. The minimum concentration of the sorbitol was 0.025 per cent.

A medium containing 3 per cent inositol, 0.05 per cent sorbitol, and 0.5 per cent yeast extract was found to support growth of *A. suboxydans* even after subculturing the organism every twenty-four hours in this medium over a period of fourteen months. Since a culture of the organism in this medium, when transferred to inositol-yeast extract medium, failed to grow in or to oxidize the inositol in this latter medium, the *A. suboxydans* cultured in a solution containing 3 per cent inositol, 0.05 per cent sorbitol, and 0.5 per cent yeast extract was used as inoculum for subsequent experiments.

*B. Development of Optimum Cultural Conditions*

The effects of varying surface-volume ratios, incubation temperatures, hydrogen ion concentrations, salt concentrations, and inositol concentrations were studied. The optimum surface-volume ratio was 1.15. The optimum temperature was 28° C. The optimum pH of the medium was 5.1 to 6.8. The presence of primary potassium phosphate retarded the initial rate of the fermentation, but did not lower the final yield of the fermentation product. The presence of 0.01 to 0.3 per cent sodium chloride decreased both the rate of fermentation and the yield of the fermentation product. The rate of reaction and the final yield of the reaction product were direct functions of the amount of yeast extract present in the medium and inverse functions of the concentration of inositol.

*C. Characteristics of the Growth Factor*

The relative stimulating effect of reagent sorbitol and the residual sorbitol in a sorbitol-yeast extract medium which had been fermented by *A. suboxydans* was ascertained. This was accomplished by diluting this culture, after centrifuging out the bacteria, with sterile water to give solutions of definite sorbitol concentrations. Portions of these sterile solutions were then added to inositol-yeast extract medium to give specific sorbitol concentrations which were the same as the reagent sorbitol concentrations in previously prepared inositol-sorbitol-yeast extract media. In equal concentrations, the sorbitol from the two sources produced the same effect. Therefore, no material was produced in the fermentation of sorbitol by *A. suboxydans* which acted as a growth factor or a stimulant for the oxidation of inositol by the organism.

The nutritive factor which is necessary in the inositol-yeast extract



medium for oxidation of the inositol by the organism was extracted from an evaporated culture of *A. suboxydans* grown on sorbitol-yeast extract medium by alcohol, but not by acetone or ether. This nutritive factor was not precipitated from a water solution by mercuric acetate. It was stable at twenty pounds steam pressure.

Erythritol, glycerol, glucose, and mannitol are as effective as sorbitol as the supplementary substrate in the inositol-yeast extract medium. Ascorbic acid, ethanol, glycine, and the calcium salts of acetic, fumaric, glutaric, and succinic acids may not be substituted for sorbitol in this medium.

#### D. *Effect of Sorbitol on the Fermentation of Other Sugars and Sugar Alcohols*

The presence of sorbitol in erythritol, glycerol, and mannitol media does not greatly affect the optimum concentrations of these substrates in regards to the activity of *A. suboxydans*. The presence of sorbitol does not enable *A. suboxydans* to ferment rhamnose, rhamnitol, or dulcitol.

#### E. *Separation and Chemical Characteristics of the i-inositol Fermentation Product*

The inositol fermentation product was separated from the culture medium by treating the medium with lead acetate, filtering, removing the excess lead as the sulfide, and then distilling the filtrate so obtained *in vacuo* to crystallization.

The evidence from the analysis of the dinitrophenylhydrazone and the acetyl derivative, from the molecular weight determination, and from the alkaline iodine absorption indicate the inositol fermentation product to be a di-keto-inositol.

# STUDIES ON THE FERMENTATIVE ACTIVITY OF YEAST ZYMIN<sup>1</sup>

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Received July 19, 1939

The data presented in the thesis represent an extension of the work of Stavely, Fulmer and Christensen (1935) on the effect of certain salts, and of ethanol, upon the fermentative activity of yeast zymín. The salts studied in detail are ammonium chloride and magnesium sulphate, which the above authors found to have special properties toward zymín. The following is a summary of the principle findings during the present research.

Ammonium chloride and magnesium sulphate accelerate the fermentative activity of maceration extract of yeast in a manner entirely analogous to their effects upon yeast zymín. The maceration extract was prepared in the usual manner by macerating a quantity of dried yeast with water at 25° C. The filtration or centrifugation of this yielded a clear extract which was used in the above mentioned experiment. These results show that the salt effect does not involve the cell wall, but the action is upon the enzyme system in the cell.

The zymíns used in the experimental work were normally prepared by desiccating fresh yeast with two successive treatments of acetone for ten and two minutes respectively and finally removing most of the acetone by a single treatment with ether. The zymín was then dried at 45° C. for 24 hours. Certain variations in this procedure were tried, and it was found that vacuum drying at room temperatures produced a product greater in activity than that dried at 45° C. Similarly, a shorter period of contact with acetone did not cause improper desiccation but did increase the zymín activity somewhat.

The zymíns prepared by the above method from yeasts from various sources were found to exhibit considerable variation, not only in fermentative activity but also in response to ammonium chloride and magnesium sulphate. When fresh yeast was stored at 5° C. the zymín prepared therefrom varied widely with the time of storage. Moreover, this phenomenon varied with the yeast employed. By taking advantage of the above facts, zymíns of various degrees of activity were prepared from the same yeast or from different yeasts. There was a distinct correlation between the activity of zymín, the degree of stimulation by ammonium chloride and the optimum concentration of the salt; the lower the activity of the zymín the greater the stimulation and the higher the optimum concentration of the salt. The effect of magnesium sulphate is practically independent of the activity of the zymín.

Similar to ammonium chloride, a series of mono-, di-, and tri-methylammoniumchlorides caused a stimulation of zymín in a manner analogous to the inorganic salt. However, as the number of methyl groups were increased, the stimulation became less pronounced. The order of stimulation was as follows:  $\text{NH}_4^+ > \text{CH}_3\text{NH}_3^+ > (\text{CH}_3)_2\text{NH}_2 > (\text{CH}_3)_3\text{NH}^+$ .

<sup>1</sup> Original thesis submitted October, 1938. Doctoral thesis number 492.

A mixture of a zymin of low activity with a highly active zymin gives a resultant rate of fermentation which is higher than would be expected on an additive basis. When a yeast, which normally gives a zymin of low activity, is washed with water, the zymin prepared therefrom shows an increased fermentative ability. A similar treatment of a low activity zymin very considerably increases its activity. The above facts point definitely to an inhibiting factor present in the fresh yeast which is carried over in the preparation of the zymin. The procedure for the separation of the inhibitor is as follows: a suspension of 25 grams of zymin in 75 cubic centimeters of tap water; this treatment removed the coenzyme the inhibitor being non-dialyzable. Filtration or centrifugation of the non-dialyzable material resulted in a clear solution which markedly inhibited the fermentative activity of a highly active zymin. The inhibitor was destroyed by heating for ten minutes at 70° C. or by standing for five minutes at room temperature at a pH of 10. The inhibitor appears to be identical in the above properties with a factor found in live yeast by Euler (1935).

Solutions of the inhibitor from zymins ranging from low to high activity showed a good correlation between the amount of inhibitor and the activity of the zymin. The addition of small amounts of ammonium chloride tends to nullify the action of the inhibitor. The above facts indicate that the degree of stimulation of various zymins by the above salt is associated with its effect upon the inhibitory substance present in the zymin.

Studies on the effect of ammonium chloride and magnesium sulphate upon the fermentation of certain intermediate products of alcoholic fermentation, showed that both salts stimulate only the reaction in which the dextrose is esterified with phosphate. Ammonium chloride not only stimulates the normal fermentation but also exerts a protective action against the toxic effect of ethyl alcohol.

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# EFFECT OF TREATED FATS ON VITAMIN A POTENCY<sup>1</sup>

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Received July 19, 1939

Since the destruction of vitamin A is of paramount concern in evaluating diets and in formulating healthy regimens, it was deemed advisable to investigate the stability of this fat-soluble vitamin in different sources and its susceptibility to destruction both in the diet and in the animal. Of particular significance in vitamin assays is the relative importance of such factors as the actual concentration of the vitamin, the extent of anti- and pro-oxygenic activity exerted by the vitamin-A-bearing oil and by other dietary constituents, the effect of storage conditions, and the form in which the vitamin activity occurs. Increased knowledge of the qualitative and quantitative nature of these factors are necessary for a rigid control of biological experiments.

This investigation was undertaken with the following aims:

1. To determine the effect of various treatments (e. g., heat, aeration, storage, etc.) upon the ability of fats to decrease the vitamin A activity of certain foods.

2. To study the role of anti-oxygens in the prevention of vitamin A inactivation.

3. To investigate the action of finely divided solids upon vitamin A activity.

4. To examine the chemical and physiological properties of treated fats, exclusive of their action on vitamin A; and, if such treatment rendered them toxic, to identify the toxic principle and study its relationship to vitamin A inactivation.

A number of experiments were performed to ascertain to what extent the destruction of vitamin A activity by heated fats depends upon the variety of the fat heated, and to what extent this destruction is influenced by the nature of the vitamin-A-bearing oil. A one-eighth inch layer of the fat was heated at 102°-105° C. for 24 hours.

It was found that oils belonging to the semi-drying or drying types, such as Mazola, cod liver oil, and cottonseed oil, upon being heated, inactivated cod liver oil; while heat failed to produce the power to inactivate cod liver oil in non-drying oils such as lard and butterfat. Most of the heated fats that were studied inactivated butterfat; one sample of heated coconut oil failed to do so. In view of the present indication that the vitamin functions in the body as an ester, it is not improbable that the vitamin's stability in cod liver oil is attributable to its occurrence as an ester; such a mode of combination may cause the vitamin to be more resistant to heated fats.

The data indicated that the saturated fatty acids, stearic and palmitic, and the glycerol portion of a fat were not responsible for the inactivating potency of heated fats; the unsaturated fatty acids, e. g., oleic, linoleic, linolenic, etc., were involved in the response of fats to heat. It was also

<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 537.

evident that the susceptibility of an oil to oxidation was dependent upon its degree of unsaturation, the type of acid responsible for the unsaturation, and the kind and concentration of anti-oxygens in the fat.

Heated Mazola inactivated butterfat even when the ration was kept at 1° C., but the inactivation was slower than that observed at 24°-30° C. Although the heated fats contained high concentrations of peroxides, the inactivating power of the fats was not strictly proportional to their peroxide content.

Different fats were heated (102°-105° C.) for varying lengths of time and at different depths and tested in feeding experiments. The vitamin-A-bearing oil was mixed in the ration, fed separately, or fed in conjunction with the fat under investigation. Butterfat and cod liver oil were used as sources of vitamin A. The concentration, both of the vitamin-A-bearing oil and the fat under examination, was altered and an investigation was made of the quantitative relationship between the two fats, as measured by the destruction of vitamin A activity and the toxicity of the heated-fat diets. Fats were autoclaved at 15 pounds for 15 minutes and then tested for their inactivating properties as well as for their response to heat. Water was incorporated into rations to discover whether it prevented the destructive action of heated fats.

Autoclaved lard did not inactivate butterfat nor did autoclaving alter the response of the fat to heat; the autoclaved lard upon being heated inactivated butterfat, in the same manner as the unautoclaved lard when subjected to similar heat treatment. The response of fats to heat, i. e., the inactivating power of heated fats, increased with the surface exposed to air and with the length of the heat treatment; the rate at which inactivating ability developed varied with the fat employed. The destruction of vitamin A activity in heated-fat diets was apparently not brought about by the heated fat alone; the decomposition products of the vitamin-A-containing oil also participated.

Heated fats appeared to be non-toxic upon ingestion since rats receiving heated-fat diets, but supplied with the vitamin-A-bearing oil apart from the remainder of the diet, remained alive; the slow growth of these animals as compared with the animals receiving the corresponding unheated-fat diets was no doubt the result of a lower food consumption of the less palatable heated-fat ration. The heated fats did not destroy vitamin A activity when the vitamin-A-bearing oil was fed separately. Water did not protect vitamin A activity against the action of heated fats. The vitamin-A-inactivating power of heated fats was not produced by heat alone but by the combined action of heat and oxygen; heat acted mainly to accelerate the action of oxygen.

A number of substances were tested for anti-oxygenic properties with a variety of fats. Both the concentration of the anti-oxygen and the severity of the heat treatment were altered. The nature of anti-oxygenic action, the resistance of this action to increasing heat, and the time at which the protection operates in relation to the heat treatment were investigated.

Anti-oxygens were found to vary in their efficacy with the fat employed; thymol was an excellent anti-oxygen with lard but did not possess anti-oxygenic properties with Mazola or soybean oil. Anti-oxygens protected the induction period but were ineffective if added after the termina-

tion of this period. Their activity diminished as oxidative conditions were intensified.

A study was made of the action of finely divided solids, such as fuller's earth and Norit A, upon butterfat. The solids did not inactivate butterfat.

Lards from hogs fed different concentrations of soybeans were given to rats to test the fats for susceptibility to oxidation in the mixed ration. Soybeans in the diet of hogs, although they increased the iodine number of the hog fat, did not markedly increase the susceptibility of the lard to oxidation.

Fats with and without thymol were stored for varying periods and at different temperatures and then tested in feeding experiments for their inactivating properties. Most of the stored fats did not inactivate butterfat; peanut oil, rancidified at room temperature, destroyed the vitamin A activity of butterfat. It was also found that no advantage was to be obtained by incorporating thymol in concentrations up to one per cent, when storing lard for 500 days at room temperature (24°-30°C.)

An experiment was conducted to determine the effect of baking upon the vitamin A content of cookies containing various fats. The results of this experiment indicated that baking did not inactivate butter in the cookies; this was true whether the cookies contained butter as the only fat or butter and some other fat such as lard, Clix, or Crisco.

Mazola and cod liver oil were aerated at room temperature and tested in feeding experiments for vitamin-A-inactivating power. Neither of the aerated oils inactivated butterfat.

The toxicity of heated fats, stored fats, heated esters, and aerated fats was tested by injecting them intraperitoneally into rats. Heated fats and heated esters proved toxic when injected intraperitoneally into rats; the latter class of compounds was the more toxic of the two. Some aerated fats and some stored fats were toxic when injected intraperitoneally; others were not. Some fats gave a positive Kreis test and yet were non-toxic to rats upon intraperitoneal injection.

Heated Mazola and heated ethyl oleate were introduced into rats by stomach tube; the corresponding unheated fat and ester were similarly given to animals. Whereas the heated fat and ester were definitely toxic to rats when injected intraperitoneally, they were only slightly so when given the animals by way of stomach tube. The unheated fat and unheated ester were non-toxic whether injected intraperitoneally or introduced by stomach tube.

Attempts were made to regenerate heated fats by agitation with fuller's earth, by steam distillation, and by treatment with semicarbazide. Heated Mazola was not regenerated by agitation with fuller's earth or by steam distillation at 100° C. Semicarbazide hydrochloride detoxified heated fats but failed to reduce their inactivating properties; ketones or aldehydes must be responsible for the toxic properties but not for the inactivating power of heated fats.

Various fats, heated for different lengths of time and stored for varying periods, were analyzed for iodine number, free fatty acid concentration, and peroxide value. The free fatty acids increased and the iodine number decreased upon heating and/or storing of the fat. The peroxide value increased to a maximum and then decreased upon heating and/or storing of the fat.



# EFFECT OF GROWTH OF MICROORGANISMS ON ACID NUMBERS OF FAT IN CREAM AND BUTTER<sup>1</sup>

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Received July 19, 1939

Changes occurring in the fat of butter are of great importance from the standpoints of flavor and keeping quality of the product. Hydrolysis of the fat may set free some of the lower fatty acids, particularly butyric, caproic and caprylic, causing a condition commonly referred to as rancidity, which is one of the most serious defects occurring in butter. Rancidity frequently develops in samples of commercial butter when they are subjected to keeping quality tests.

Many microorganisms are able to hydrolyze butterfat. Organisms of this type are widespread in nature, often being present in raw cream, water and dairy plant equipment. They are ordinarily killed by pasteurization but recontamination after pasteurization may occur. If such organisms gain entrance to pasteurized cream in sufficient numbers and find conditions suitable for growth, they may cause serious defects in the resulting butter. Salt retards the growth of these organisms, so that they produce the most serious defects in unsalted butter.

After subjecting good quality, unsalted commercial butter from many sources to keeping quality tests, rancidity was a common defect. All of the samples were made from pasteurized cream. Since lipase is readily inactivated or destroyed by heat, and since there was little chance for contamination of the cream with the enzyme after pasteurization, the rancidity which developed probably was the result of the growth of microorganisms. Most of the lipolytic organisms commonly found in milk and cream are readily destroyed by ordinary pasteurization temperatures, therefore the organisms responsible must have gained entrance subsequent to pasteurization. Very few of the samples of salted butter observed became rancid when subjected to keeping quality tests. The salt evidently was very inhibitory to the organisms responsible for the rancidity, since the opportunities for contamination of salted butter were essentially the same as with the unsalted butter.

The acid numbers of the fat of the fresh, unsalted samples were uniformly low, usually less than 1.0. After holding at 21° C. for 6 days, many of the samples showed increased acid numbers. However, there was no definite correlation between the acid numbers of the fat and the quality of the butter after storage. While high acid numbers usually accompanied the development of rancidity, rancid samples with low acid numbers were sometimes encountered. Conversely, samples not showing rancidity frequently had relatively high acid numbers. In the rancid samples with low acid numbers on the fat, the proportions of the total fat acid that were volatile, while often unmeasurable by the method employed, probably were considerably higher than in the rancid samples with relatively high acid numbers.

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<sup>1</sup> Original thesis submitted June, 1939. Doctoral thesis number 522.

Lipolytic organisms varied considerably in their ability to produce rancidity in unsalted butter. Some organisms which definitely caused hydrolysis of fat, as evidenced by increased acid numbers, failed to cause a rancid condition. *Oospora lactis*, for example, greatly increased the acid numbers in the fat in cream or in butter and yet, in some instances, a rancid flavor did not develop. Three bacterial species produced rancidity regularly even with very slight increases in the fat acid values. Differences in the proportions of total acid that were volatile as a result of the growth of the various organisms were significant and accounted for the conditions mentioned.

Lactic acid in cream in amounts greater than are normally present, either produced by the growth of the common lactic organisms or added directly, had no effect on the acid number of the fat. The acid was completely absent from the fat after churning, apparently being left in the buttermilk. Acid tended to inhibit the growth of the lipolytic organisms but was not effective in controlling them. Ripening cream to a relatively high acidity for unsalted butter no doubt aids in the control of certain organisms. This practice, however, cannot be expected to prevent growth of the undesirable types.

The growth of organisms in unsalted butter was somewhat more limited than in cream. In butter, the food supply was not as plentiful as in cream and because of the physical structure of butter the organisms were somewhat confined and were less able to migrate to new food supplies. The organisms studied, however, were all very detrimental to the quality of unsalted butter stored at temperatures as low as 5° C.

The neutralizing process, as applied to sour cream in the manufacture of butter, was definitely effective in lowering the acid number of the fat. This indicates that the alkali not only neutralized the water soluble fatty acids that were in the serum but also partially neutralized the acids present in the fat. The fact that the fatty acids in the cream fat are largely neutralized during the processing of the cream preparatory to churning eliminates the possibility of a good correlation between the acid number of the fat of butter and the quality of the cream from which it was made.

In trials in which *O. lactis* was grown in cream or butter, the percentage of the total acid liberated that was volatile was very small. Certain organisms, particularly *O. lactis*, were capable of growing in a medium in which salts of the lower fatty acids provided the sole source of carbon. It is probable that *O. lactis* largely consumed the volatile acids liberated from the fat. However, it is possible that this organism may have exerted a selective action on the fat, liberating only the higher acids, and that it would not have consumed the lower fatty acids in the synthetic media if other food materials had been available. With other organisms grown in cream or butter, the ratios of volatile to non-volatile acids liberated were very uniform in all trials with the same organisms, which indicated that the fat hydrolysis proceeded in a definite manner with each organism. This relationship prevailed in all trials with an organism regardless of varying growth conditions or the degree of fat hydrolysis produced.

In commercial unsalted butter showing rancidity, this same uniform volatile non-volatile acid relationship was very evident, regardless of the degree of rancidity. In all of those samples the percentages of the total acids in the fat that were volatile were comparatively high. This might indicate that bacteria were chiefly responsible for the rancid condition in

commercial unsalted butter, since *O. lactis* and *Mycotorula lipolytica* in pure cultures both produced relatively low volatile acid values.

Butterfat alone was not a suitable food for microorganisms, for even when partially emulsified with water and inoculated with lipolytic organisms, no acid number increases resulted after 30 days at 21° C.



# THE QUANTITATIVE SPECTROGRAPHIC ANALYSIS OF SOILS<sup>1</sup>

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Received July 19, 1939

The purpose of this investigation was to develop a method for the analysis of soil solutions using a d-c carbon arc as the spectroscopic source. The method used is described as the internal standard method (2). Analyses were made by comparing the intensity of a test element line to the intensity of an internal standard line, both of which were recorded on the same photographic plate. This comparison was made by calculating the ratio of the deflections of the galvanometer of a non-recording microphotometer. The intensity ratio of the selected pair of lines was determined for a series of solutions in which the amount of the test element was varied, and from this data the working curve (1) for the test element was prepared. Several series of standard solutions were made in which the percentage composition of the test elements to be determined was varied over a wide range. Each of these solutions contained Mo as an internal standard and Na as a spectroscopic buffer. Sufficient Na and

TABLE 1. *Lines used and range of concentrations covered in the analysis of soil solutions*

| Element  | Spectral lines       |         | Range of analysis<br>%                  % |        |
|----------|----------------------|---------|---|--------|
|          | Test<br>element<br>A | Mo<br>A |   |        |
| Si ..... | 2881                 | 3112    | .00005                                    | — .008 |
| Si ..... | 2881                 | 3132    | .0003                                     | — .01  |
| Si ..... | 2881                 | 3170    | .0003                                     | — .01  |
| Al ..... | 3092                 | 3112    | .0005                                     | — .04  |
| Al ..... | 3092                 | 3132    | .001                                      | — .50  |
| Al ..... | 3092                 | 3170    | .001                                      | — .50  |
| Al ..... | 3944                 | 3903    | .01                                       | — .30  |
| Al ..... | 3961                 | 3903    | .01                                       | — .30  |
| Fe ..... | 3020                 | 3112    | .0001                                     | — .01  |
| Fe ..... | 3020                 | 3132    | .001                                      | — .09  |
| Fe ..... | 3020                 | 3170    | .001                                      | — .09  |
| K .....  | 3217                 | 3132    | .001                                      | — .10  |
| K .....  | 3217                 | 3170    | .001                                      | — .10  |
| Ca ..... | 3933                 | 3903    | .0001                                     | — .15  |
| Mg ..... | 2795                 | 3132    | .0001                                     | — .10  |
| Mg ..... | 2795                 | 3170    | .0001                                     | — .10  |

Mo were added to the soil solutions to make their concentration the same as in the standard solutions.

Spectroscopic carbon electrodes of the highest purity, prepared by the method of Duffendack and Wolfe (1), were used. These rods were

<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 530.

cut at right angles to their length, the ends were made perfectly flat and smooth, and the corners were rounded off with a file. A drop of the solution to be analyzed was transferred to the end of the positive electrode by means of a glass rod and the electrode was then dried quickly in an oven.

The arc was excited by means of a 500 volt storage battery with a ballast resistance of ten cone-type heater units wired in series with a 50 ohm rheostat. An arc gap of .75 mm. and a current of 2.5 amperes were adopted as standard.

The instrument used throughout this investigation was a Bausch and Lomb medium quartz spectrograph. The spectra were recorded on Wratten and Wainwright panchromatic plates and were developed for 3 minutes at 18° C. For development two parts of water and one part of fresh Eastman D-72 were used.

The lines used and the range of concentration of the elements quantitatively determined are shown in table 1. The range of analysis listed does not, in any sense, indicate the upper or lower limit of possible determinations by the spectrographic method. They do indicate, however, the range which was investigated by the writer.

The data available indicate that for the determination of Si, Al, Fe, Mg, K, and Ca the accuracy is between 10 and 15 per cent. Greater accuracy than this might be attained if the technique were applied to the routine analysis of soil solutions, but the 10 to 15 per cent accuracy is a tolerable figure for a method using a d-c arc.

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# USE OF FUNCTIONALS IN OBTAINING APPROXIMATE SOLUTIONS OF LINEAR OPERATIONAL EQUATIONS<sup>1</sup>

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Received July 19, 1939

Many problems in mathematics concern special cases of a general linear operational equation. Several of the methods used in solving these special problems are examples of the more extensive method used in this paper to obtain solutions of the general linear operational equation.

The use of functionals in obtaining an approximation of the exact solution is explained, ways for estimating the error involved are explored, relations of the method used to existing methods are given, and ways in which extensions can be made are indicated.

The general linear operational equation has the form  $LU = P$  in the matrix notation which is used throughout this paper for generality. The  $m \times 1$  matrix  $P$  with functions as elements is given, the unknown  $n \times 1$  matrix  $U$  with functions as elements is to be determined, and the  $m \times n$  matrix  $L$  with linear operators as elements is given. The elements of the matrix  $L$  commute with constants and transform to zero expressions vanishing identically. In homogeneous problems the matrix  $P$  is zero.

Examples of the problem  $LU = P$  are systems of differential and integral equations, expansions of functions in terms of known functions with constant coefficients, certain problems in indefinite integration, boundary value problems, difference equations, etc. in which the unknown occurs linearly. Characteristic values may appear in the problem.

The unknown  $U$  is replaced by an expansion in terms of known functions. In matrix notation this expansion is a product  $VA$  of a known matrix  $V$  of expansion functions by an unknown constant matrix  $A$  and there is obtained  $L(VA) = P$ . It is the sense of the present method of approximation that, while the foregoing equation usually is not exactly true, the constant matrix is determined by an equation of the form

$$F(L(VA)) = FP$$

in which  $F$  is a functional. The product  $VA$  is obtained as an approximation for the unknown  $U$ .

Because it can be verified easily by actual expansion that the usual associative property holds for the matrix products under consideration, parentheses are unnecessary.

The difference  $P - LVA$  is the value of a remainder  $J$ . This remainder is such that, operated upon by the functional selected, the product  $FJ$  vanishes. Knowledge of the character of the functional matrix can indicate the properties of the remainder. It is possible to study the remainder before or after the actual solution for  $A$ , because it is determined by the choice of  $L$ ,  $P$ ,  $V$ , and  $F$  and is expressible as the quotient of two determinants with known elements.

Ways of measuring several kinds of error involved in the approxi-

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<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 532.



mation are in terms of expressions for the error permitted by the functions of expansion for  $U$  and for  $P$ . If there exists an expansion  $V\alpha$  satisfying the relation  $U = V\alpha + \varepsilon$ , it is related to the expansion  $VA$  obtained by functional methods by the following equations:

$$\begin{aligned} A - \alpha &= (FLV)^{-1}FL\varepsilon, \\ VA - V\alpha &= V(FLV)^{-1}FL\varepsilon, \\ P - LVA &= L\varepsilon - LV(FLV)^{-1}FL\varepsilon. \end{aligned}$$

Although it is assumed here for simplicity that the inverse  $(FLV)^{-1}$  exists, analogous results are obtained when it does not. Similarly, when there exists an expansion  $LV\alpha$  satisfying the relation  $LV\alpha + \bar{\varepsilon} = P$ , the corresponding equations

$$\begin{aligned} A - \alpha &= (FLV)^{-1}\bar{F}\bar{\varepsilon}, \\ VA - V\alpha &= V(FLV)^{-1}\bar{F}\bar{\varepsilon}, \\ P - LVA &= \bar{\varepsilon} - LV(FLV)^{-1}\bar{F}\bar{\varepsilon}, \end{aligned}$$

are true. It is evident that an exact answer is obtained by the use of functionals, if the functions of expansion are sufficient for the representation of an exact answer. When the problem involves characteristic values, a way of estimating the error is to determine how well the approximation satisfies the equation defining the problem.

For the successful application of the methods of Ritz, Trefftz, Bousinesq (least squares), Krawtchouk (Kravchuk), perturbation theory, and expansions, it is necessary that only a single element of  $P$  be different from zero. The present method includes each of these as a special case by an appropriate selection of the functional  $F$ .

If the correct linear combinations of the elements of one functional permitting a solution are selected as elements of another, application of the latter results in biorthogonalization,

$$FLVA = IA = A = FP.$$

A generalization of the orthogonalization of functions to the method of functionals is possible and more valuable than biorthogonalization. The addition of a linearly independent function for the expansion of  $P$  necessitates the calculation of only the additional constant coefficient. The initial elements of  $A$  are obtained with relative rapidity.

Arbitrary parameters can be made to appear in the approximation by including them either in the functions of expansion or in the functionals or in both. The parameters then can be chosen to minimize an expression selected for the measurement of error. If either or both of the substitutions  $V + \kappa\bar{V}$  for  $V$  and  $F + \nu\bar{F}$  for  $F$  are made, the solution for  $A$  proceeds in the usual way. A means of giving the functions of expansion and the functionals variations is initiated.

If a constant matrix  $V$  approximating the unknown  $U$  is written as  $IV$  in which the unit matrix  $I$  is a known matrix of expansion functions and the unknown constant matrix  $V$  is to be determined, the method of difference equations, Fredholm's solution of integral equations, and an extension of the latter are included in the present method. If, on the other hand, the functional  $F$  is replaced by a more general linear operator, the solution for  $A$  can frequently be carried out. Agreement with the method of successive approximation is obtained by such a procedure. The Neumann series solution of the problem  $(L - \lambda\bar{L})U = P$  is found as an example.

# EFFECT OF ETHER ON THE TOXICITY OF CERTAIN FUMIGANTS TO THE CONFUSED FLOUR BEETLE, *TRIBOLIUM* *CONFUSUM* DUVAL

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Received July 19, 1939

The purposes of this research are (1) to determine the dosage-mortality relations toward the confused flour beetle (*Tribolium confusum* Duval) of ether, carbon disulfide, carbon tetrachloride and ethyl acetate at 30 degrees C. and an exposure time of two hours, and (2) to investigate the synergistic or antagonistic effect of ether on carbon disulfide, carbon tetrachloride and ethyl acetate.

The 2-hour dosage-mortality curves of ether, carbon disulfide, carbon tetrachloride and ethyl acetate at 30 degrees C. against the confused flour beetle are presented. The median lethal concentrations of these gases are 832 mg. per liter, 115 mg. per liter, 135 mg. per liter and 108 mg. per liter respectively, indicating that carbon disulfide, carbon tetrachloride and ethyl acetate are approximately 6 to 8 times as toxic to this insect as ether at the 50 per cent point.

From the dosage-mortality curve for each gas, the concentrations killing 15, 25 and 35 per cent of the test insects were read. Each gas was then admixed with concentrations of ether such that the combined toxicity of each mixture should cause theoretically 50 per cent mortality. In all cases the per cent mortality obtained was far greater than that expected. Carbon disulfide-ether mixtures and carbon tetrachloride-ether mixtures gave mortalities approaching 100 per cent, while ethyl acetate-ether mixtures gave mortalities of about 80 per cent.

The effect of certain sublethal concentrations of ether upon the toxicity of carbon disulfide, carbon tetrachloride and ethyl acetate to the confused flour beetle was also studied. From the results obtained in this phase of the investigation it is indicated that the toxicity of carbon disulfide and of carbon tetrachloride is directly increased by sublethal concentrations of ether. The toxicity of ethyl acetate, however, is decreased as the sublethal concentration of ether is increased.

The results of this investigation lead to the conclusion that ether, a substance of relatively low toxicity, may be used to synergise certain gases (carbon disulfide, carbon tetrachloride and ethyl acetate) of comparatively much higher toxicity. In lower concentrations, however, ether may be used to antagonize ethyl acetate and actually decrease its toxicity to the confused flour beetle.

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<sup>1</sup> Original thesis submitted May, 1939. Doctoral thesis number 509.

# RELATION OF THE STRUCTURE OF SUGARS TO THE CHEMISM OF THE BUTYL-ACETONIC FERMENTATION<sup>1</sup>

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Received July 19, 1939

Most of the fundamental investigations of the butyl-acetonic fermentation have been limited to studies of the fermentation of starch in corn meal. This limitation is due principally to the fact that corn meal is practically the only carbohydrate-containing material that has been employed in commercial scale fermentations. The dissimilation of corn mash produces the neutral products, *n*-butyl alcohol, acetone and ethyl alcohol, commonly called 'solvents', in the approximate ratio of 60:30:10, respectively.

Peterson, Fred and Schmidt (1) studied the fermentation of the pure sugars, glucose, xylose and arabinose, from the standpoint of yields of neutral products. Glucose and xylose were found to give approximately the same ratio of solvents as are obtained from corn mash, but the yield of acetone from arabinose was appreciably higher. Underkofler, Christensen and Fulmer (2) found that the fermentation of carbohydrates, starch, dextrose, maltose, levulose, sucrose and xylose produced the neutral products in essentially the normal ratio of 60:30:10. The fermentation of *l*-arabinose, described by Underkofler and Hunter (3), gave a ratio of 50:40:10. The investigation herein reported was concerned with the relation between the configuration of several carbohydrates and the yields of the solvents produced in the butyl-acetone fermentation.

A modification of the Shaffer and Somogyi (4) micro-method of analysis for reducing sugars was developed to meet the need for rapid, routine sugar determinations. The reagent as developed was capable of being used for sugar solutions containing up to 10 mg. of reducing sugar in a 5 ml. sample. Micro-methods previously described were designed for sugar concentrations only one-fifth as great. For a determination, 5 ml. each of the reagent and of the solution to be analyzed were heated in a boiling water bath for a length of time sufficient to give practically maximum reduction. For glucose, sorbose and *d*-arabinose 20 minutes in the water bath were found to be sufficient. Mannose, galactose, *l*-arabinose, maltose and lactose were heated 30 minutes while the slow reducing rhamnose required 40 minutes. After cooling the heated solutions, the cuprous oxide formed in each was determined by acidifying with sulfuric acid and titrating the excess iodine liberated with standard sodium thiosulfate. For determinations of reducing sugars in a bacterial culture, the removal of proteins was found to be unnecessary.

Some factors which influence the yields of neutral products in semi-synthetic media were studied. The yields of solvents were found to decrease with decreasing volumes of medium when contained in flasks of such size as to hold as large a quantity as practicable. The yields of acetone decreased more sharply with decreasing volume, that is, with in-

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<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 528.



creasing surface-volume ratio, than did yields of butyl alcohol and ethyl alcohol. Saturation of corn mash with carbon dioxide, preliminary to inoculation, resulted in slightly lowered yields for all surface-volume ratios employed.

Optimum concentrations for corn-gluten meal, soy-bean meal and bacto-peptone as nutrient materials in a semi-synthetic medium containing glucose were determined. The maximum yields of solvents were obtained for each nutrient, when 100 ml. of medium contained, respectively, 1.5 grams of corn-gluten, 0.5 grams of soy-bean meal or 0.75 grams of peptone. Corn-gluten meal gave the highest yields of products and peptone the lowest. No solvents, whatsoever, were formed from glucose in the presence of bacto-yeast extract in any of the several concentrations in which this material was employed as a nutrient. Yeast extract proved to be definitely inhibitory to the fermentation when used in combination with other nitrogenous substances. Normal yields were not obtained from glucose in media containing only peptone as nutrient material. The yields of acetone and ethyl alcohol were more markedly decreased than those of butyl alcohol in peptone media in comparison with normal yields in media containing corn-gluten meal.

The course of the fermentations of the carbohydrates, glucose, mannose, galactose and lactose, along with corn meal for control, was followed by means of determination of pH, total acidity, quantity of reducing sugar remaining and of the formation of individual solvents. Mannose and glucose were readily fermented giving normal yields of each of the three neutral products. The dissimilation of mannose was more rapid than that of glucose but no more complete. Lactose was fermented less rapidly and less completely than glucose with consequent lower yields. About 80 per cent of this disaccharide was utilized. At the end of the fermentation of lactose the proportion of butanol was appreciably higher and ethanol lower than the normal, while that of acetone was the same as from glucose or corn mash. Galactose gave low yields with relatively high proportions of butanol and low proportions of acetone and ethanol. The fermentation stopped when only one-third of the sugar disappeared. The total acidity increased continuously throughout the fermentation period. The final total yield of solvents from galactose actually utilized was 25 per cent while the yields from glucose, mannose and lactose were approximately 36 per cent, calculated on the same basis. The data obtained for the fermentation of these carbohydrates are summarized in table 1. The action of the butyl organism on the sugars, sorbose, rhamnose, *d*-arabi-

TABLE 1. Yields of solvents from fermentation of various carbohydrates

| Substrate       | Percentage total yield | Percentage yield from sugar utilized | Final proportions of individual solvents |       |      |
|-----------------|------------------------|--------------------------------------|--|-------|------|
|                 |                        |                                      | But.                                     | Acet. | Eth. |
| Corn mash ..... | 32.6                   | —                                    | 58                                       | 30    | 12   |
| Glucose .....   | 34.5                   | 35.7                                 | 57                                       | 28    | 15   |
| Mannose .....   | 33.1                   | 35.2                                 | 58                                       | 30    | 12   |
| Galactose ..... | 8.5                    | 25.2                                 | 70                                       | 25    | 5    |
| Lactose .....   | 28.5                   | 35.9                                 | 63                                       | 31    | 6    |

nose and glucose and on the sugar alcohol, sorbitol, when used to replace equivalent quantities of corn meal in varying increments, was investigated. None of these substances, except glucose, was dissimilated in any of the varying degrees of replacement. None of the polyhydric alcohols, perseitol, *i*-inositol and *l*-arabitol were attacked in semi-synthetic media containing corn-gluten.

The pentose sugar, *d*-lyxose, was prepared by the degradation of calcium galactonate with hydrogen peroxide. Attempts to ferment lyxose, when contained in a sirup from which the sugar was not crystallized, were not successful. The conclusion that this compound is not fermentable is not fully justified because of the possible presence of toxic impurities in the sirup.

Dihydroxyacetone, when added to sterile semi-synthetic medium at the time of inoculation was partially fermented; about one-sixth of this compound was utilized as determined by reducing sugar analysis. Dihydroxyacetone, when added to the medium before sterilization, underwent changes during the heating which rendered the medium unfermentable.

The data obtained in this study, together with those obtained by previous investigators, indicate that the configuration of a sugar most favorable toward fermentability by the butyl-acetone organism consists of a pair of *cis* hydroxyl groups located in the molecule adjacent to a primary alcohol group. While such an arrangement holds in general for the sugars, it fails for some of the sugar alcohols. Sorbitol, perseitol and arabitol, each containing a pair of *cis* hydroxyl groups adjacent to a terminal carbon atom, were not found to be fermentable. The fact that *l*-arabinose was readily fermented while *d*-arabinose was unattacked makes it apparent that stereoconfiguration is of prime importance.

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# A CLASSIFICATION OF THE PROTEOLYTIC MICROCOCCI ISOLATED FROM DAIRY PRODUCTS<sup>1</sup>

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Received July 19, 1939

In studying changes in dairy products, bacteriologists have given the micrococci relatively little attention since it is generally thought that these organisms produce changes slowly and therefore are of little importance. The usual classifications of the micrococci are based on such characteristics as gelatine liquefaction, nitrate reduction and the fermentation of various carbohydrates. These characteristics are of little practical value to the dairy bacteriologist. The action of the organisms on the constituents of milk, however, may be of considerable importance.

Various micrococci found in milk and milk derivatives are capable of attacking the proteins of milk. The extent to which the milk is proteolyzed varies greatly with the particular organism. In some instances proteolysis can be detected by the appearance of the milk while in other instances chemical analyses are necessary to detect changes.

The ability of certain micrococci to break down proteins is important since many dairy products contain enough proteins to make such changes undesirable from the standpoint of quality; however, such changes may be of importance in cheese ripening. The micrococci, therefore, should be seriously considered by the dairy bacteriologist.

This study was undertaken with a three fold objective: First, to isolate proteolytic micrococci from various dairy products and to study their general characteristics; Second, to determine the effect of various types of proteolytic micrococci on milk and butter; and Third, to classify the proteolytic micrococci in dairy products and to identify them.

By plating on milk agar and incubating at 37° C. for 48 to 72 hours, proteolytic micrococci were readily isolated from many samples of milk, butter, cheese and ice cream. They were obtained from 340 of 580 samples of milk, from 36 of 42 samples of butter, from 20 of 36 samples of cheese (cheddar) and from 30 of 46 samples of ice cream.

On the basis of chromogenesis, the proteolytic micrococci were readily divided into four groups—red, orange, yellow and white (non-pigmented). The red micrococci were not studied in detail because of the small numbers encountered.

Studies on the influence of various factors on chromogenesis indicated:

- (a) That when grown in an atmosphere of carbon dioxide, the general pigment production on agar was not significantly different than when grown in air, although with a few of the orange cultures the pigment was lighter in color.
- (b) That when grown on potato, some of the orange organisms produced a deeper color than when grown on agar.

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<sup>1</sup> Original thesis submitted June, 1939. Doctoral thesis number 523.



- (c) That the pH value of the medium had an effect on the pigment production, a value of 7.0 being more favorable than values of 6.6 or 7.6, although pigment production was definite at all three values.
- (d) That holding organisms in sterile water for 6 days before inoculating on agar did not influence pigment production.
- (e) That subjecting cultures to 55° C. for 10 minutes before inoculating on agar had little or no effect on pigment production.
- (f) That more highly colored pigment was produced at 21° C. than at 37° C. with the orange cultures but not with the yellow cultures.
- (g) That agar cultures held in darkness for 10 days were not as deeply pigmented as when held in a sun lighted room.

Milk cultures of the proteolytic micrococci were viable for 3 to 4 months and agar slant cultures for 10 to 12 months at room temperature.

When litmus milk was inoculated with the various organisms, four general types of changes were produced while a few cultures produced no noticeable change.

The titratable acidity produced in milk by 20 representative cultures varied from 0.12 to 0.66 per cent, calculated as lactic acid. Organisms of the orange and white groups generally gave higher values than those of the yellow group.

All of the cultures studied grew well on beef infusion agar slants and in beef broth.

A majority of the cultures studied reduced nitrates.

In general, the organisms did not assimilate amino nitrogen, although ammonia nitrogen was assimilated by many cultures of the orange and yellow groups but by none of the white group. All cultures were capable of producing ammonia from peptone, whereas none produced ammonia from nitrates.

All cultures except a few of the white group liquefied gelatine.

Cultures of the yellow group generally produced larger amounts of carbon dioxide than cultures of the orange or white groups.

Hemolysis was shown by some cultures of the orange and yellow groups but by none of the white group.

Lipolysis was limited to organisms of the orange group. None of the yellow or white organisms studied were lipolytic, according to the methods used.

Diastatic action was exhibited by 1 culture of the orange group and by 96 of the yellow group. None of the white cultures produced diastase.

In general, most of the cultures studied fermented dextrose, galactose, lactose, levulose and maltose. Dextrine, glycerol, mannitol, salicin, sorbitol, sucrose and xylose were fermented by some cultures. Arabinose and raffinose were not fermented.

In broth cultures all of the organisms were destroyed at temperatures ranging from 55° to 65° C. for 15 minutes.

Appreciable amounts of acetylmethylcarbinol plus diacetyl or volatile acid were not produced by the organisms. Tests for hydrogen sulphide and indol were regularly negative.

Thirty representative cultures were found to be non-pathogenic when inoculated into guinea pigs intraperitoneally.

None of the organisms studied produced spores, and all were apparently non-motile.

Protein breakdown in skimmilk by four representative cultures of proteolytic micrococci was studied in detail. When grown in milk at 37° or 21° C. for 2 to 7 days, the organisms increased the amount of nitrogen in the serum, although the extent of proteolysis varied considerably with the cultures. In general, the fractions of nitrogen soluble in trichloroacetic acid, soluble and insoluble in ethyl alcohol or insoluble in phosphotungstic acid increased during the incubation, whereas the fractions insoluble in trichloroacetic acid or soluble in phosphotungstic acid remained about the same. The amounts of amino nitrogen increased in the serum during the holding.

The general action of 38 cultures of proteolytic micrococci on unsalted butter was studied by churning small portions of sterile cream inoculated with relatively large numbers of the organisms, and then examining the butter after various periods at 4° and 20° C. Definite defects were produced in many samples of butter after 4 to 8 weeks at 4° C. or 1 to 5 weeks at 20° C. Certain cultures of the orange group produced rancid, cheesy and pungent flavors, while certain of the yellow group produced cheesy, musty or bitter flavors. In general, members of the orange group changed the flavor of butter more rapidly and more extensively than members of the yellow or white groups.

An attempt was made to identify, on a species basis, the 426 cultures of proteolytic micrococci. The organisms were separated into 15 species of the genus *Micrococcus* on the basis of chromogenesis, action on nitrates, action on milk and the fermentation of various carbohydrates. Ten species were identified from descriptions given by other investigators, while 5 species were unidentified. A key to the identification of the 15 species of proteolytic micrococci is given.

# THE DETERMINATION OF ELASTIC CONSTANTS BY PIEZO-ELECTRIC METHODS<sup>1</sup>

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Received July 19, 1939

The relationships existing between the elastic constants and the frequencies of the natural modes of vibration of a finite plate of homogeneous material have never received a rigorous formulation. On the other hand, the solution of the relationships between elastic constants and natural frequencies of an infinite plate is rigorous.

In the evaluation of the elastic constants of a material, an infinite plate can be approximated, either by making use of a large, thin plate or by using harmonics of the fundamental modes of vibration. The latter method was used in this investigation because of its greater accuracy.

When a plate is vibrating with a fundamental mode or a harmonic of this mode, the wave planes of the standing waves which are produced are parallel to the surface of the plate. Thus, as the order of the harmonic becomes progressively higher, more wave planes are crowded between the surfaces of the plate, and so the size of the plate becomes larger in comparison with each wave length, approximating ever closer the theoretical infinite plate.

The six independent adiabatic elastic constants of quartz were evaluated by frequency measurements of piezo-electrically excited harmonic vibrations of finite quartz plates placed in a filter circuit in a specially designed apparatus. The apparatus consisted of a stable oscillator having a frequency range of about 300 to 36,000 kilocycles per second, a temperature chamber for maintaining the plates at a constant temperature, an audio amplifier for the approximate location of resonance points of the quartz plates, a sensitive vacuum tube voltmeter for the accurate location of resonance points, and a frequency meter having an accuracy such that frequencies could be determined by its use to one part in twenty-five thousand.

The approximation of the behavior of the finite plates to the behavior of infinite plates of the same thickness was excellent for harmonics of higher order than the thirtieth for the particular plates used. The frequencies of harmonics up to the eighty-seventh were measured for the main modes of vibration, and up to the two hundred and sixty-third harmonic for certain lateral modes.

The quartz plates were cut from two well-developed, flaw-free quartz crystals. An optical method which made use of light reflected from the facets of the natural crystals, was used to get the orientation of the plates correct within one minute of the specified angle. The two surfaces of the plates were ground plane and parallel to each other within one ten-thousandth centimeter.

All angles of the plates were accurate right angles and opposite sides were parallel to four significant figures. Four useful plates were cut from

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<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 526.



each of the natural crystals, these plates having the following orientations:  $0^\circ, 0^\circ$ ;  $30^\circ, 0^\circ$ ;  $0^\circ, 45^\circ$ ; and  $30^\circ, 38^\circ 12'$ . The mathematical relationships between the elastic constants and the natural frequencies of vibration for infinite plates of each of these orientations were derived and these relationships were used in the evaluation of the constants.

The results obtained with similarly-oriented plates from the two natural quartz crystals were the same within the experimental error of the thickness measurements of the plates. This fact is an indication of the uniformity of all flaw-free quartz.

The adiabatic elastic constants of quartz as evaluated by this method are:  $C_{11} = 87.45 \times 10^{10}$ ,  $C_{12} = 6.03 \times 10^{10}$ ,  $C_{14} = 17.8 \times 10^{10}$ ,  $C_{44} = 57.1 \times 10^{10}$ ,  $C_{13} = 14.4 \times 10^{10}$ ,  $C_{33} = 109.0 \times 10^{10}$ , dynes per square centimeter.

# RADIANT HEAT TRANSFER IN CERAMIC KILNS<sup>1</sup>

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Received July 19, 1939

A survey of the literature indicates that no attempts have been made to employ the information on the radiant heat transfer from non-luminous gases, used in the design of heat treating furnaces and pipe stills, to effect an improvement in ceramic firing practices. While numerous heat balances have been run on various types of kilns and the results of many published, no effort has been made to determine the mechanism by which the heat present in the products of combustion is transferred to the product in these kilns, and it has been popularly believed that convection is the important mechanism.

While this is probably true at low temperatures, the work of Schack (1) in Germany and of Hottel (2) and his co-workers in the United States shows that at elevated temperatures heat radiated by water vapor and by carbon dioxide accounts for more than 90 per cent of the total heat transferred. These investigators were concerned with furnaces in which there could be only superficial chemical reactions between the products of combustion and the material being heated, while in ceramic firing practice such reactions may be of considerable importance. It therefore was considered desirable to ascertain if existing firing practices in the ceramic industry could be improved by the addition of water vapor and carbon dioxide to the products of combustion without effecting undesirable chemical reactions between them and the ware.

In order to fire rapidly, a small muffle furnace lined with high-temperature, light-weight refractory, was built. The muffle was made of silicon carbide and closed at both ends by refractory plates through which were inserted sillimanite tubes for introducing and exhausting the gases in which the samples were fired. The gas streams were broken up and diffused by an appropriate set of baffles.

Gases were introduced into the muffle at slightly greater than atmospheric pressure in order that any leakage might take place from the interior of the muffle outwards, thereby protecting the samples being fired from contact with the products of combustion. Samples cut from dry but unfired commercial hollow tiles from five Iowa plants were measured, weighed, and set in the muffle. Firings were made to various temperatures according to a fixed schedule in three atmospheres, air, carbon dioxide, and water vapor. During firing, a temperature of  $\pm 1.5^{\circ}$  C. was the maximum variation permitted and duplicate firings were made in almost all cases. The purity of the atmosphere inside the muffle was checked by analysis during firing and samples were held at predetermined temperatures for thirty minutes. If the temperature varied more than three degrees Centigrade from the fixed schedule during the firing, the run was discarded. Temperature measurements were made with a pre-

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<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 525.

cision potentiometer and chromel-alumel thermocouples whose cold junctions were maintained at 0° C. by means of an ice bath.

The porosity of each fired sample was determined by means of a Washburn-Bunting porosimeter and a mercury volumeter. The percentage of ferrous iron in each sample was determined by chemical analysis and the carbon dioxide content was determined for all samples fired in carbon dioxide atmospheres.

In each of the clays used in this investigation it was found that at any temperature the porosity of the sample fired in water vapor was less than that of one fired in carbon dioxide or in air, and that the porosity of a sample fired in carbon dioxide was less than that of one fired in air. On a basis of radiant heat transfer considerations alone, it is to be expected that at any temperature a sample fired in carbon dioxide would have a lower porosity than one fired in water vapor. The converse, however, appears to be true.

The results obtained in this investigation can be explained by postulating that chemical combination between the fluxes in the clay and the carbon dioxide of the atmosphere removed enough of the flux from active action that the firings in water vapor and in carbon dioxide atmospheres are not completely comparable. It is possible also that water vapor acts as a catalyst in promoting the action of the fluxes present. With carbon dioxide and with water vapor atmospheres it is impossible to determine to what extent the decrease in porosity from firing in air at any temperature is a consequence of greater heat transfer and to what extent this decrease is attributable to reduction.

It was found by analysis that a considerable part of the flux naturally present in each of the clays had been converted to carbonates by chemical reaction with the carbon dioxide in the atmosphere. It was further discovered that all samples fired in either carbon dioxide or in water vapor were not completely oxidized and analysis for ferrous iron in the samples so fired showed that the amount of reduction as measured by the percentage of ferrous iron in the fired sample was inversely proportional to the porosity of the sample. Since reduction did take place the value of porosity as a measurement of maturity is somewhat doubtful but it seems probable that the firing in water vapor resulted in a lower porosity at any temperature than can be attributed to reduction alone.

It may be possible to take advantage of the radiant heat transfer from water vapor and carbon dioxide in ceramic firing without injury to the product, by replacing a portion of either gas with oxygen or air. To what extent this replacement is necessary remains to be determined.

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# THE ACCURACY OF THE PLATING METHOD FOR ESTIMATING THE NUMBER OF BACTERIA, ACTINOMYCES AND FUNGI IN A LABORATORY SAMPLE OF SOIL<sup>1</sup>

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Received July 19, 1939

Fourteen hundred and sixty-five samples of field soil, held in the laboratory one day after crushing, were plated by the recognized technique in four replicates of one dilution for counts of fungi and of a higher dilution for counts of bacteria and of actinomyces during the crop season of 1936, 1937, and 1938. A  $X^2$  value was calculated for each set of counts. These values for each group of microorganisms were distributed into classes and the number in each class compared with the theoretical for the Poisson series. The data for each year indicate that the fungal counts conform to expectancy on the basis of random sampling and prove that the method provides an accurate estimate of the population in the dilution plated. Too many sets of counts of bacteria in each year yield high  $X^2$  values. The counts of actinomyces conform to expectancy and may be accepted as accurate for the dilution plated.

In an attempt to determine the cause of this abnormality for sets of counts of bacteria, samples were plated on the afternoon of the day taken from the field. Three hundred and four samples plated in six replicates of one dilution and another 100 samples plated in four replicates yield  $X^2$  values, whose distribution conform to expectancy. Accordingly, the plate method provides an accurate estimate of the bacterial population of soil in the dilution plated if the procedure is carried out within six hours after sampling. Data on 88 samples plated on the day of sampling, on 88 samples held one day, on 88 samples held two to five days and on 88 samples held eight to thirteen days show that the discrepancy between the actual and theoretical distributions of  $X^2$  values becomes progressively greater at each successive period of holding the samples. Further, the data indicate that the area sampled, the season, the medium used and the technique of plating bear no relation to the abnormal variation in counts of bacteria on replicate plates.

A record was kept of the presence of abnormal types of bacterial colonies and various genera of fungi on all plates from 468 samples plated one day after sampling and crushing during 1938. The data show that sets having pin-point colonies or spreading colonies of the Mucorales on one or more plates usually have high  $X^2$  values. Counts on such plates should be excluded from the estimate of the mean number of bacteria in the sample. Likewise, the number of actinomyces colonies on each plate from these samples was recorded. The  $X^2$  values for these counts were found to conform to expectancy, indicating that the factor or factors associated with a large number of high  $X^2$  values for counts of bacteria does not affect the count of actinomyces in the same way.

Percentage moisture and P values corresponding to the  $X^2$  values

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<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 495.

for the counts of bacteria obtained each year were correlated. The data yield coefficients that are not significant in each case.

Six 25 gram aliquots taken from a well mixed laboratory sample were plated, each in six replicate dilutions with four replicate plates from each dilution, for counts of fungi. The analysis of variance shows that there are not significant differences in counts of fungi among replicate dilutions from one aliquot, but that there are among aliquots from one laboratory sample. Each replicate dilution was raised and the final dilutions plated in four replicates for counts of bacteria. The data for bacterial counts show significant differences among dilutions from one aliquot, but not among aliquot samples.

In a second experiment, one 25 gram aliquot taken from a sample was diluted 1:10 and another 25 gram aliquot was diluted 1:50. Each original dilution was raised to 1:5,000 in eleven replicate dilutions, which were plated in four replicates for fungi. The experiment was repeated ten times. In this case, the data show that the 1:10 method of making the original dilution yields significant differences among the final dilutions and that the 1:50 system, which reached 1:5,000 in one transfer, is preferable. Each dilution was raised to 1:500,000 and the final dilutions were plated for bacteria in six replicates. The analysis shows that the 1:10 method is not reliable because of significant differences among dilutions and that the 1:50 method is preferable, although failing to reduce the differences to insignificance.

The 1:50 and 1:100 systems of making the original dilution were compared in experiment 3, as well as differences among aliquot samples. A fresh sample was plated in five aliquots for each system, each aliquot in ten replicate dilutions and each dilution in four replicate plates for bacteria. The 1:50 system again shows significant differences among dilutions and the 1:100 system is not preferable. Likewise, there are significant differences among aliquot samples in each case.

In experiment 4 all dilutions were raised from 1:50 original dilutions. Each trial consisted of six aliquots, raised in six replicate series of dilutions and plate in six replicate plates from each final dilution. This was repeated four times for fungal counts and six times for counts of bacteria. The analysis again shows that for fungal counts differences among dilutions are not significant, while for bacterial counts they are. Again, there are significant differences for aliquot samples in the case of both fungal and bacterial counts.

In experiments 2, 3 and 4 the plating, pouring, piling of plates in the incubator and counting of plates were carried out in one order. The analysis shows that none of these practices adds anything significant to the error of plating.

Since the errors of the sample used and of the dilution plated are significant, valid information on the counts of bacteria, actinomyces or fungi in a laboratory sample cannot be obtained by the procedure of using one 25 gram sample and one final dilution from it, regardless of the number of replicate plates made from the dilution. By using six aliquot samples with three replicate dilutions from each and one plate for each dilution the estimate would be based upon these three factors in about their proportionate weight.

Only by carefully designed experiments and the application of statistical methods to check the validity of the results obtained can progress

be made in developing the plate method of counting bacteria or fungi in soil to a stage where it may be used for practical application to the problems of agriculture.



# INSECTICIDAL ACTION OF SOME SUBSTITUTED PYRROLIDINES<sup>1</sup>

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Received July 19, 1939

A series of  $\alpha$ -substituted pyrrolidines was synthesized and the dissociation constants of the new compounds measured with a hydrogen electrode. These constants placed the thienyl and mesityl radicals in the electron sharing ability curve<sup>2</sup>, the thienyl radical being located half-way between the phenyl and the o-chlorophenyl radicals, and the mesityl at about the same value as the o-chlorophenyl. The dissociation constants are given in table 1.

TABLE 1

| Compound  | Elec | E.M.F. | K <sub>B</sub>        | — log K <sub>B</sub> |
|---|------|--------|-----------------------|----------------------|
| $\alpha$ -Thienyl- $\alpha$ -pyrrolidine .....<br>(in H <sub>2</sub> O)   | A    | .7991  | $2.24 \times 10^{-5}$ | 4.65                 |
|   | B    | .7991  | $2.24 \times 10^{-5}$ | 4.65                 |
| $\alpha$ -Thienyl- $\alpha$ -pyrrolidine .....<br>(in CH <sub>3</sub> OH) | A    | .6049  | $3.39 \times 10^{-7}$ | 6.47                 |
|   | B    | .6049  | $3.39 \times 10^{-7}$ | 6.47                 |
| 2-Mesityl- $\alpha$ -pyrrolidine .....<br>(in CH <sub>3</sub> OH)         | A    | .7062  | $1.86 \times 10^{-7}$ | 6.73                 |
|   | B    | .7062  | $1.86 \times 10^{-7}$ | 6.73                 |

For the insecticidal studies the common firebrat, *Thermobia domestica* Pack., was selected as the test insect. Groups of ten insects were sprayed with a series of concentrations by means of a modified Tattersfield apparatus<sup>3</sup>. This consisted of an atomizer located in the top of a bell jar so as to center the spray on a 10 cm. crystallizing dish. The atomizer was connected to an air pressure line through a Hoke regulator set to maintain a constant pressure of 9 lbs. per square inch. This apparatus has the advantage of requiring only small amounts of material for the tests.

Because of the slight solubility of most of the compounds in water a fifty per cent (by volume) acetone solution was used as the sprayin medium. The acetone lowered the surface tension to a point where it was unnecessary to use any spreading agent, and eliminated any errors likely to occur from the toxic effect of a spreader.

Stock solutions of four grams per 100 cc. of solution were made up, and from these the lower concentrations made by dilution. Those concentrations in the center portion of the mortality curve were sprayed five or six times, while those near the ends were sprayed in duplicate or triplicate.

<sup>1</sup> Original thesis submitted June, 1939. Doctoral thesis number 519.

<sup>2</sup> Hixon and Johns. J. Am. Chem. Soc., 49:1786 (1927); Starr, Bulbrook, and Hixon, *ibid.*, 54:3971 (1932); Goodhue and Hixon, *ibid.*, 56:1329 (1934).

<sup>3</sup> Craig and Richardson, Iowa State Coll. J. Sci., 7:477 (1933).

Mortality counts were made at the end of twenty-four hours since test runs indicated no variation in the count over a period of forty-eight hours. All paralyzed insects were counted as dead.

The effect of hydrogenation was determined by comparing the pyrrolidines with the corresponding pyrrolines, and in all cases, except with the thienyl compounds, the reduced compounds were less toxic than the corresponding pyrrolines. The discrepancy in the case of the thienyl radical can be accounted for on the basis of physical properties.

No general relationship could be discovered between toxicity and dissociation constants. Although the most toxic compounds tested were also the most negative in nature, the more positive compounds like the cyclohexyl and butyl were more toxic than would be indicated by a comparison of dissociation constants.

Unexpected results were obtained in comparing the insecticidal action of optically active isomers. Levo and dextro-cyclohexylpyrrolidine had identical toxicities, but in turn were more toxic than the racemic compound. A similar result was obtained with the phenyl isomers. Thus the toxicity of the racemic compound is not the additive effect of the two component isomers.

In table 2 are listed the concentrations in grams per 100 cc. and also mols per 100 cc. to give a 50 per cent mortality. These values were taken from curves obtained by plotting the logarithms of the concentrations against the per cent mortality expressed in probit units.

TABLE 2. Comparative toxicities of certain alpha-substituted pyrrolines and pyrrolidines

| Compound   | Conc. in<br>gms. per 100<br>cc. for 50 per<br>cent mortality | Conc. in mols<br>per 100 cc. for 50<br>per cent mortality |
|--|--|---|
| 1- $\beta$ -Pyridyl- $\alpha$ -N-methylpyrrolidine (nicotine) .. | .25  | .0015   |
| 2-Mesityl- $\alpha$ -pyrrolidine .....                           | 1.0  | .0053   |
| $\alpha$ -Thienyl- $\alpha$ -pyrrolidine .....                   | 1.2  | .0079   |
| $\alpha$ -n-Butylpyrrolidine .....                               | 1.9  | .0148   |
| dl- $\alpha$ -Cyclohexylpyrrolidine .....                        | 1.5  | .0098   |
| l- $\alpha$ -Cyclohexylpyrrolidine .....                         | .95  | .0065   |
| d- $\alpha$ -Cyclohexylpyrrolidine .....                         | .95  | .0065   |
| dl- $\alpha$ -Phenylpyrrolidine .....                            | 2.0  | .0131   |
| l- $\alpha$ -Phenylpyrrolidine .....                             | 1.3  | .0089   |
| d- $\alpha$ -Phenylpyrrolidine .....                             | 1.3  | .0089   |
| 2-Mesityl- $\alpha$ -pyrroline .....                             | .8   | .0043   |
| $\alpha$ -Cyclohexylpyrroline .....                              | 1.0  | .0066   |
| $\alpha$ -n-Butylpyrroline .....                                 | 1.4  | .0111   |
| $\alpha$ -Phenylpyrroline .....                                  | 1.4  | .0097   |
| $\alpha$ -Thienyl- $\alpha$ -pyrroline .....                     | 1.4  | .0093   |

# RELATIONSHIP BETWEEN THE ELECTRON-SHARING ABILITY OF RADICALS AND THE ASSOCIATION OF ORGANIC COMPOUNDS<sup>1</sup>

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Received July 19, 1939

It is known from the work of Hixon and Johns<sup>2</sup> and their collaborators that one order or series of the known organic radicals is sufficient to correlate the acid and basic properties of each member of numerous families of organic compounds. This series which is an arrangement of the radicals in the order of their electron-sharing ability has been shown to have numerous other applications to organic chemistry especially in the field of reversible reactions. Those radicals which cause an increase in the acidic properties of a compound when substituted in the molecule in place of hydrogen have been given positive values of electron-sharing ability and vice versa. By certain theoretical considerations, involving the fact that the hydrogen atom attached to oxygen is the controlling element in both molecular dissociation and association, the author was able to predict that the entry of radicals of high positive electron-sharing ability into an associating body should cause a decrease in the molecular complexity of the compound. The predicted effect was experimentally shown for the carboxylic acids of the type  $\text{R COOH}$  where "R" varied from the methyl to the trichloromethyl radical. The measurements were made by vapor density methods.

Data were also obtained for the same series of acids in solvents such as acetone, diethyl ether, and methyl acetate. Here, too, the association was found to vary inversely with the electron-sharing ability of the substituted radical although the effect was quite small. The data were obtained by measurements upon the vapor pressures of the solutions concerned. A brief review of the literature is given that shows that this effect of electron-sharing ability upon association is quite general and that it applies to classes of compounds other than the carboxylic acids. The effect of quantum mechanical resonance in stabilizing association structures is briefly discussed as are the effects of temperature, steric hindrance, chelation and other factors in producing phenomena of greater magnitude that obscure and overcome the generally small effect of electron-sharing ability.

In a study and resume of the theories regarding the effect of the solvent in influencing the association of the solute, the author arrived at the conclusion that the older theory which regards the dielectric constant as the controlling factor could not be retained in the light of the more modern theories of valency. As an alternative he would suggest (as have other recent investigators) that the reaction or possible reaction of the solvent in solvating the solute should be the controlling factor and that for the same conditions of temperature and of concentration this one fac-

<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 504.

<sup>2</sup> Hixon and Johns, *J. Am. Chem. Soc.*, **49**:1786 (1925).



tor should be sufficient to explain all solvent action upon association regardless of the values of the dielectric constants concerned. By this hypothesis the solvation reaction would be of the same nature as the association reaction and the same factor (i. e. electron-sharing ability) should control both. Approximate calculations were made to show the mechanism of the solvent effect by this hypothesis and a derivation made of the solvating order of a series of solvents of the type  $C_6H_5R$ . This derived order was found to be essentially the same as that determined experimentally by previous investigators.

In considering the effect of the electron-sharing ability of the radicals concerned upon the solvation reaction the conclusion was reached that the entry of radicals of high electron-sharing ability into a solvating compound should cut down the extent of the solvation reaction and that this would be reflected by the increase of the associating power of this compound as a solvent. An attempt was made to demonstrate this effect by determining the molecular weight of a standard solute (benzoic acid) in a series of substituted solvents of the same chemical type. Due to the uncontrollable factors entering into the determinations, such as temperature differences, etc., the results were not interpreted too closely but it is felt that the role of electron-sharing ability in controlling the solvation reaction has been demonstrated.

Various errors and inconsistencies in the literature (notably the case of benzoic acid in water) have been pointed out and corrected.

Various applications of electron-sharing ability to other fields closely related to association have been pointed out. A brief discussion of each case from the standpoint of the literature has been given.

# ORGANOMETALLIC COMPOUNDS OF GROUP VIII ELEMENTS<sup>1</sup>

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The first organometallic compound of the group VIII elements to be prepared was trimethylplatinum iodide<sup>2</sup>. With trimethylplatinum iodide as a starting material, a series of trimethylplatinum compounds was prepared. The trimethylplatinum compounds are generally crystalline solids which have no melting points. The preparation of phenyliron iodide and ethyliron iodide has been reported from the action of phenylzinc chloride and ethylzinc iodide, respectively, on ferrous iodide<sup>3</sup>.

The present investigation deals with the attempted preparation of characteristic organometallic derivatives of the group VIII elements in an effort to determine some of their properties.

Iron, cobalt, and nickel show no tendency for direct union with organic halides to form organometallic compounds.

Iron halides, with the exception of ferric fluoride, react with phenylmagnesium halides with the formation of metallic iron and biphenyl in quantitative yields. Cobalt and nickel halides react in the same manner. No evidence of organometallic compounds is found in these reactions. Ferric fluoride reacts with phenylmagnesium bromide with the formation of benzene. No evidence of the formation of an organoiron compound is obtained.

$\alpha$ -Naphthylmagnesium bromide and  $\alpha$ -naphthyllithium give low yields of bi- $\alpha$ -naphthyl in reactions with ferrous chloride and ferrous iodide. No evidence is obtained for the formation of an organoiron compound in these reactions. Addition of benzyl bromide, prior to hydrolysis, gives no ketone.

The reaction of phenylzinc chloride with ferrous iodide proceeds to the formation of biphenyl and metallic iron. The reaction proceeds very slowly but without any evidence of the formation of phenyliron iodide. Hydrolysis of aliquots from the reaction mixture at intervals shows the formation of biphenyl to be proceeding regularly as the proportion of ferrous hydroxide to zinc hydroxide increases in the hydrolysis products. The solution obtained from addition of ferrous iodide to phenylzinc chloride, followed by two hours refluxing, is of the same order of chemical reactivity as phenylzinc chloride. The addition of benzoyl chloride gives benzophenone while allyl bromide gives no reaction on standing sixteen hours with either phenylzinc chloride or the reaction mixture of phenylzinc chloride and ferrous iodide.

The reaction of ethylzinc iodide with ferrous iodide proceeds slowly. Evolution of gas from the reaction is slow but continuous. Six hours refluxing of a solution containing 0.1 mole of ethylzinc iodide and 0.1 mole

<sup>1</sup> Original thesis submitted July, 1938. Doctoral thesis number 480.

<sup>2</sup> Pope and Peachey, *J. Chem. Soc.*, 95:571 (1909); *Proc. Chem. Soc.*, 23:86 (1907).

<sup>3</sup> Champetier, *Bull. soc. chim.*, [4] 47:113 (1930). Job and Reich, *Compt. rend.*, 174:1358 (1922); *Bull. soc. chim.*, 32:1390 (1922).

of ferrous iodide in ether produced 575 ml. of gas which analyses indicated as a mixture of ethylene, ethane, and butane. Hydrolysis of the reaction gave a mixture of ferrous hydroxide and zinc hydroxide which contained 39.4 per cent of ferrous hydroxide and 60.6 per cent zinc hydroxide.

Tetraethyllead reduces ferric chloride promptly and completely to ferrous chloride.

Ferrous iodide reacts promptly with tri-*p*-anisyllead in ether-benzene solution with precipitation of lead iodide and tetra-*p*-anisyllead. Hydrolysis of the solution gives a mixture of organolead compounds consisting chiefly of di-*p*-anisyllead diiodide. No evidence of an organoiron compound in the reaction mixture is obtained. The reaction proceeds in the same manner in the presence of iron powder.

Cobaltous bromide gives a 20 per cent yield of bimesityl when reacted with 2,4,6-trimethylphenylmagnesium bromide.

Phenylmagnesium iodide reacts with ferrous chloride, cobaltous bromide, nickelous bromide, ruthenium trichloride, rhodium trichloride, and palladium chloride in an ether-benzene solution at  $-10^{\circ}$  C. with the formation of metal and biphenyl in quantitative yields. Osmium trichloride under the same conditions produces biphenyl in a 53 per cent yield. No organoosmium compound is isolated. Iridium trichloride reacts very slowly with phenylmagnesium iodide to produce a 28 per cent yield of biphenyl. A small amount of an amorphous brown solid is obtained which burns leaving a residue of iridium metal. Ten equivalents of phenylmagnesium iodide are required to produce a solution which persists in giving a color test in the reaction of phenylmagnesium iodide with anhydrous platinic chloride. An insoluble white complex is formed which generates an amorphous, brown, organic, platinum and iodine containing solid on hydrolysis. This solid consists of a mixture of phenylplatinum compounds from which no individual compounds have been isolated. This solid is only slightly soluble in benzene, alcohol, or chloroform. It is readily soluble in dioxane. Various fractions are obtained which vary in platinum content from 30.3 per cent to 47.8 per cent.

Methylmagnesium iodide reacts with platinous chloride with the formation of a difficultly soluble, amorphous, brown powder. Analysis of this substance corresponds to the calculated value for dimethylplatinum diiodide.

$\alpha$ -Naphthylmagnesium bromide reacts with platinous chloride with the formation of di- $\alpha$ -naphthalplatinum. This substance is obtained as a brown powder which is very soluble in benzene or chloroform. It is very slightly soluble in alcohol, ether, or petroleum ether (b. p.,  $60-68^{\circ}$ ). Di- $\alpha$ -naphthylplatinum acts as a condensing agent for the preparation of 2,4-dimethylbenzophenone from benzoyl bromide and *m*-xylene. Platinic chloride also acts as a condensing agent for this reaction. The yields of 2, 4-dimethylbenzophenone are 70 to 80 per cent of theoretical, with only a very small amount of platinum compound being required.

The reaction of methylmagnesium iodide with anhydrous platinic chloride gives 40 per cent yields of trimethylplatinum iodide. A trace of tetramethylplatinum is also formed in the reaction. The remainder of the reaction products consists of a mixture of highly iodinated compounds. A small amount of a black crystalline material is obtained which is soluble in water and ethyl acetate. Analyses for platinum and iodine indi-



cate that this compound is methylplatinum pentaiodide. Amorphous, difficultly soluble, materials corresponding in platinum and iodide content to dimethylplatinum diiodide and methylplatinum triiodide are obtained.

Hexamethyldiplatinum results by the action of metallic potassium on trimethylplatinum iodide in boiling benzene. Hexamethyldiplatinum is a colorless, crystalline solid which is easily soluble in ether, acetone, or benzene. It explodes sharply on heating. It reacts with iodine in ether to give trimethylplatinum iodide.

Tetramethylplatinum is obtained from trimethylplatinum iodide and methylsodium in hexane. Tetramethylplatinum is a colorless solid which is readily soluble in benzene, acetone, ether, and petroleum ether (b. p. 60-68°). This compound is unaffected in twenty-four hours by iodine in boiling chloroform. Hydrogen chloride cleaves tetramethylplatinum to trimethylplatinum chloride on heating for fifteen to twenty minutes in boiling petroleum ether (b. p., 60-68°).

None of the platinum compounds obtained have melting points.

# THE CONDUCTANCE OF SOLUTIONS OF ORGANOSUBSTITUTED AMMONIUM CHLORIDES IN LIQUID HYDROGEN SULFIDE<sup>1</sup>

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Received July 19, 1939

Organic ammonium salts were found to form conducting solutions in liquid hydrides of phosphorus, sulfur, and the halogens by McIntosh and Steele (3) and in liquid hydrides of the halogens and sulfur by Steele, McIntosh and Archibald (7). Antony and Magri (1) have reported that solutions of tetramethylammonium iodide in liquid hydrogen sulfide conduct. Quam and Wilkinson (5) have reported the conductances of solutions of methylammonium, dimethylammonium, and triethylammonium chlorides at a number of different concentrations in liquid hydrogen sulfide. Their work shows distinctly the increased conductance of solutions of substituted ammonium chlorides as the number of the substituents increases. Their report indicates also an increased molecular conductance with increasing concentration.

## THE INVESTIGATION

Conductance measurements were made on solutions of mono-, di-, tri-, and tetramethylammonium chlorides and the corresponding ethyl and n-propyl compounds dissolved in pure liquid hydrogen sulfide maintained at constant temperature with a mixture of solid carbon dioxide and ether. The conductance bridge used was designed according to that described by Shedlovsky (6) using 5000-ohm ratio arms. Wire wound resistors were used in measuring high resistances up to  $10^6$  ohms. Resistances above  $10^6$  ohms were calculated from the measured parallel resistance of the conductance cell and a resistor. The 5000-ohm ratio arms were replaced by a Kohlrausch slide wire when measuring resistances below 500 ohms.

The molecular conductances  $\lambda$  shown in table 1 are those read at the concentrations indicated from molecular conductance-square root of concentration plots for the several compounds.

Table 1 shows that the propylsubstituted compounds conduct better than the corresponding ethyl compounds and that they in turn conduct better than the corresponding methyl compounds. The only exception to this statement is that triethylammonium chloride shows higher values at low concentrations than those for tripropylammonium chloride. The

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<sup>1</sup> Original thesis submitted March, 1939. Doctoral thesis number 508.

TABLE 1. *Molecular conductances at selected concentrations*

| C     | $(C_2H_5)_4NCl$ | $\lambda \times 10^4$<br>$(C_2H_5)_4NCl$ | $(CH_3)_4NCl$ |
|-------|-----------------|--|---------------|
| .09   | .....           | .....                                    | .....         |
| .04   | 58,000          | 39,000                                   | 19,000        |
| .01   | 32,000          | 21,000                                   | 12,000        |
| .0064 | 31,000          | 20,000                                   | 13,000        |
| .0036 | 34,000          | 21,000                                   | 15,000        |
| .0016 | 42,000          | 27,000                                   | 18,000        |
| .0004 | 72,000          | 47,000                                   | 27,000*       |

| C     | $(C_2H_7)_3NHCl$ | $(C_2H_5)_3NHCl$ | $(CH_3)_3NHCl$ |
|-------|------------------|------------------|----------------|
| .09   | 2,300            | 1,840            | 840            |
| .04   | 1,300            | 880              | 350            |
| .01   | 700              | 560              | 160            |
| .0064 | 660              | 570              | 120            |
| .0036 | 640              | 620              | 140            |
| .0016 | 640              | 740              | 150            |
| .0004 | 690              | 1,100            | 150            |

| C     | $(C_2H_7)_2NH_2Cl$ | $(C_2H_5)_2NH_2Cl$ | $(CH_3)_2NH_2Cl$ |
|-------|--------------------|--------------------|------------------|
| .09   | .....              | .....              | 47               |
| .04   | .....              | .....              | 29               |
| .01   | 538                | 40                 | 23               |
| .0064 | 63                 | 43                 | 22               |
| .0036 | 82                 | 51                 | 22               |
| .0016 | 113                | 72                 | 32               |
| .0004 | 182                | 132                | 60               |

| C     | $C_2H_7NH_3Cl$ | $C_2H_5NH_3Cl$ | $CH_3NH_3Cl$ |
|-------|----------------|----------------|--------------|
| .09   | .....          | .....          | .....        |
| .04   | .....          | .....          | .....        |
| .01   | 5.4            | .7             | .....        |
| .0064 | 6.0            | .9             | .....        |
| .0036 | 7.1            | 1.2            | .....        |
| .0016 | 8.8            | 1.4            | .....        |
| .0004 | > 15           | .....          | .....        |

\* Estimated by graphical interpolation

tetrasubstituted compounds conduct much better than the trisubstituted compounds which in turn conduct better than the disubstituted compounds and the monosubstituted compounds yield the poorest conducting solutions. In fact no evidence for conduction was found for methylammonium chloride. Quam and Wilkinson (5) found that solutions of methylammonium chloride did conduct the current.

The compounds show in general an increasing conductance both in more dilute and in more concentrated solutions. Those which do not, the monosubstituted salts and dipropyl- and diethylammonium chlorides would be expected to have increasing conductance in more concentrated solutions.

#### INTERPRETATION OF RESULTS

Approximate values of the limiting conductances of the di-, tri-, and tetrasubstituted compounds were estimated using Walden's rule (9, 10). According to Walden's rule, the product of the limiting conductance of the solute and the viscosity of the solvent is constant for a given electrolyte.



The limiting conductance values of Moore and Winmill (4) in water at 25° were used in this calculation for the di- and trisubstituted compounds. Walden (9) has calculated the product of limiting conductance by viscosity for the tetrasubstituted iodides and has shown (8) that the product for the iodide ion is 0.009 unit larger than for the chloride ion.

The dissociation constant  $K$  for each salt was calculated by use of Ostwald's dilution law:

$$K = \frac{C\sigma^2}{1 - \sigma} (f^2), \text{ where } C \text{ is the concentration, } \sigma \text{ is the fraction}$$

dissociated, and  $f$  is the activity coefficient for the ions. Dissociation is so slight for these compounds that  $1 - \sigma$  is negligibly less than one. Assuming that  $f$  equals one,

$$K = C\sigma^2.$$

Since  $\sigma$  is equivalent to  $\frac{\lambda}{\lambda_0}$ ,

$$K = C \left( \frac{\lambda}{\lambda_0} \right)^2$$

The dissociation constant  $K$  calculated from values of  $\lambda$  at a concentration of  $4 \times 10^{-4}$  mols per liter are shown in table 2.

TABLE 2. *Dissociation constants*

|                   | R = methyl            | R = ethyl             | R = propyl            |
|-------------------|-----------------------|-----------------------|-----------------------|
| $R_2NH_2Cl$ ..... | $.22 \times 10^{-12}$ | $1.1 \times 10^{-12}$ | $2.4 \times 10^{-12}$ |
| $R_3NHCl$ .....   | $.12 \times 10^{-11}$ | $8.3 \times 10^{-11}$ | $3.8 \times 10^{-11}$ |
| $R_4NCl$ .....    | $.91 \times 10^{-7}$  | $3.1 \times 10^{-7}$  | $9.3 \times 10^{-7}$  |

These dissociation constants are probably not very accurate. However, the relative order of magnitude of each value is probably correct. It should be noted that a large increase in dissociation occurs upon replacing the last hydrogen of the ammonium ion with an alkyl group. Kraus (2) has called attention to this effect in solvents having no great affinity for the proton and he attributes it to "what is called a hydrogen bond."

## CONCLUSIONS

1. Organosubstituted ammonium chlorides form solutions in liquid hydrogen sulfide which conduct the electric current.

2. Increasing the size of the substituted ammonium ion by changing the substituent from methyl to ethyl to propyl groups increases the conductance of their solutions but not greatly. One exception to this statement was found. Triethylammonium chloride in solutions more dilute than 0.0036 molar forms solutions having higher molecular conductances than solutions of tripropylammonium chloride in the same concentration range. No explanation for this exception is offered.

3. Increasing the number of substituents in the ammonium ion increases the conductance significantly. The increase is especially marked on substituting the last hydrogen of the ammonium ion.

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# REACTIONS OF SOME HIGH-MOLECULAR-WEIGHT FATTY ACID DERIVATIVES<sup>1</sup>

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Received July 19, 1939

In recent years improvements in the preparation of long-chained aliphatic compounds starting from normal aliphatic acids such as lauric and stearic acids have made a number of compounds readily available for further investigation. Among these, are alcohols<sup>2</sup>, acid chlorides, and nitriles<sup>3</sup>. This thesis has been concerned with reactions of compounds of these types.

Lauronitrile on treatment with phenylethyllithium amide ( $C_6H_5NC_2H_5$ )Li,<sup>4</sup> gave 2-*n*-decyl-3-iminomyristonitrile,  $C_{11}H_{23}C \begin{smallmatrix} | \\ CN \end{smallmatrix} - CHC_{10}H_{21}$  (b. p. 230-235°/3 mm.), which on hydrolysis with

$C_{11}H_{23}C \begin{smallmatrix} | \\ HN \end{smallmatrix} - CHC_{10}H_{21}$  (b. p. 230-235°/3 mm.), which on hydrolysis with concentrated sulfuric acid gave 2-*n*-decyl-3-ketomyristamide, (m. p. 114-115°). On treating this amide with alcoholic potassium hydroxide laurone was obtained. Hydrolysis of the β-imino-nitrile with alcoholic hydrogen chloride gave 2-*n*-decyl-3-ketomyristonitrile (m. p. 44-45°).

Under exactly the same conditions as with lauronitrile, stearonitrile  $C_{16}H_{33}C \begin{smallmatrix} | \\ CN \end{smallmatrix} - CHC_{17}H_{35}$  gave 2-*n*-hexadecyl-3-iminoarachidonitrile,  $C_{17}H_{35}C \begin{smallmatrix} | \\ HN \end{smallmatrix} - CHC_{16}H_{33}$  (m.p. 54-55°)

and on treatment with concentrated sulfuric acid 2-*n*-hexadecyl-3-ketoarachidamide (m. p. 114-115°) resulted. Hydrolysis of the amide gave stearone. With alcoholic hydrogen chloride 2-*n*-hexadecyl-3-ketoarachidonitrile (m. p. 68-69°) was formed.

A series of thioamides was prepared by the thiohydrolysis of the even numbered carbon atom nitriles from lauronitrile to stearonitrile. The compounds prepared were: thiolauramide (m. p. 82-83°), thiomyristamide (m. p. 87-88°), thiopalmitamide (m. p. 93-94°) and thiostearamide (m. p. 96-97°).

By the Fries rearrangement of *p*-phenylphenyl stearate,  $C_6H_5C_6H_4OCC_{17}H_{35}$ , (m. p. 73-74°) two products were obtained;

$p$ -(*p*-hydroxyphenyl) stearophenone,  $HOC_6H_4C_6H_4CC_{17}H_{35}$  (m. p. 141-142°) and 2-hydroxy-5-phenylstearophenone,  $(C_6H_5C_6H_4OH)C \begin{smallmatrix} || \\ O \end{smallmatrix} C_{17}H_{35}$ , (m. p. 63-64°). Methylation of these products with dimethyl sulfate gave,

<sup>1</sup> Original thesis submitted July, 1938. Doctoral thesis number 481.

<sup>2</sup> Adkins, "Reactions of Hydrogen," University of Wisconsin Press, Madison (1937).

<sup>3</sup> Raston, Harwood, and Poole, J. Am. Chem. Soc., **59**: 987 (1937).

<sup>4</sup> Ziegler, Eberle, and Ohlinger, Ann., **504**: 94 (1933).



*p*-(*p*-methoxyphenyl)stearophenone (m. p. 116-117°) and 2-methoxy-5-phenylstearophenone (m. p. 53-54°). On oxidation, *p*-(*p*-methoxyphenyl)stearophenone gave terephthalic acid; identified as its dimethyl ester. The interaction of 4-methoxy-3-biphenylmagnesium bromide and stearonitrile gave 2-methoxy-5-phenylstearophenone. A Friedel-Crafts reaction between stearoyl chloride and 4-methoxybiphenyl gave *p*-(*p*-methoxyphenyl)stearophenone, which was identical with the product obtained from the Fries rearrangement after methylation.

The interaction of  $\beta$ -naphthylmagnesium bromide and stearonitrile formed  $\beta$ -stearoylnaphthalene (m.p. 65-66°). *p*-Biphenyllithium and stearonitrile gave *p*-phenylstearophenone,  $\text{C}_6\text{H}_5\text{C}_6\text{H}_4\text{CC}_{17}\text{H}_{35}$ , (m.p. 108-109°).

2-Nonacosanone,  $\text{CH}_3\text{CC}_{17}\text{H}_{35}$ , (m.p. 55-56°) was obtained from methylmagnesium bromide and stearonitrile.

A Friedel-Crafts reaction between stearoyl chloride and biphenyl, and stearoyl chloride and diphenyl ether, gave *p*-phenylstearophenone and *p*-phenoxy-stearophenone (m. p. 62-63°), respectively.

Sulfonation of *p*-phenylstearophenone formed 4'-stearoyl-4-biphenyl-sulfonic acid,  $\text{HSO}_3\text{C}_6\text{H}_4\text{C}_6\text{H}_4\text{CC}_{17}\text{H}_{35}$ , (m.p. 142-145°) which on oxidation

gave 4'-sulfo-4-biphenylcarboxylic acid,  $\text{HSO}_3\text{C}_6\text{H}_4\text{C}_6\text{H}_4\text{CO}_2\text{H}$ , (m. p. of *p*-toluidine salt, 288-289°). Sulfonation of 4-biphenylcarboxylic acid gave the same sulfonic acid, 4'-sulfo-4-biphenylcarboxylic acid, which on fusion with potassium hydroxide gave 4'-hydroxy-4-biphenylcarboxylic acid.<sup>5</sup> Decarboxylation of this acid resulted in the formation of *p*-phenylphenol (mixed m. p.). With chlorosulfonic acid, *p*-phenyl-stearophenone gave a trisulfonic acid. On oxidation, 4'-sulfo-4-biphenylcarboxylic acid was obtained.

4-stearoylphenoxybenzenesulfonic acid,  $\text{HSO}_3\text{C}_6\text{H}_4\text{OC}_6\text{H}_4\text{CC}_{17}\text{H}_{35}$ , (m. p. 95-98°) was obtained by sulfonation of *p*-phenoxy-stearophenone. Oxidation with dilute nitric acid formed 4-sulfo-phenoxybenzenesulfonic acid,  $\text{HSO}_3\text{C}_6\text{H}_4\text{OC}_6\text{H}_4\text{CO}_2\text{H}$ , (m. p. of *p*-toluidine salt 266-267°) which on fusion with potassium hydroxide gave *p*-hydroxybenzoic acid.

Hydrogenation of lauro- and stearonitriles with Adkins' copper-chromium oxide<sup>6</sup> catalyst gave di-*n*-dodecylamine (m. p. 52-53°) and di-*n*-octadecylamine (m. p. 73-74°), respectively.

*n*-Dodecyl- and *n*-octadecyl chlorides were obtained from *n*-dodecanol and *n*-octadecanol with thionyl chloride. On heating one mole of *n*-dodecyl chloride with two moles of di-*n*-dodecylamine, tri-*n*-dodecylamine was obtained (m. p. of hydrochloride 78-79°). Using the same procedure, tri-*n*-octadecylamine (m. p. 54-55°) was obtained from *n*-octadecyl chloride and di-*n*-octadecylamine. The hydrochloride (m. p. 96-97°) was obtained from the free amine.

Laurone and stearone were synthesized by heating lauric and stearic acids, respectively, with iron powder.<sup>7</sup> The reduction of myristone and stearone with sodium and *n*-butyl alcohol gave the secondary alcohols,

<sup>5</sup> Fieser and Bradsher, J. Am. Chem. Soc., 58:1738 (1936).

<sup>6</sup> Adkins, *ibid.*, 54:1138 (1932).

<sup>7</sup> Easterfield and Taylor, J. Chem. Soc., 99:2298 (1911).

14-heptacosanol and 18-pentatriacontanol, respectively. 18-Iodopentatriacontane (m. p. 43.5-45°) was obtained from 18-pentatriacontanol. Attempts to synthesize *s*-tetra-*n*-heptadecylethane from 18-iodopentatriacontane were unsuccessful. By the reduction of 18-iodopentatriacontane, *n*-pentatriacontane was obtained. Only a 22 per cent yield of *n*-tetracosane resulted from the treatment of *n*-dodecylmagnesium bromide with cupric chloride.

*n*-Octadecyl bromide was obtained from *n*-octadecanol, aqueous hydrobromic acid and concentrated sulfuric acid in 87 per cent yields.

The reaction of *n*-dodecylmagnesium bromide with laurone gave 12-*n*-

dodecyltricosan-12-ol,  $\text{C}_{11}\text{H}_{23}\overset{\text{C}_{12}\text{H}_{25}}{\underset{\text{OH}}{\text{C}}}\text{C}_{11}\text{H}_{23}$ , (b. p. 270-275°/2 mm.). The inter-

action of *n*-octadecylmagnesium bromide and stearone gave 18-*n*-octa-

decylpentatriacontan-18-ol,  $\text{C}_{17}\text{H}_{35}\overset{\text{C}_{18}\text{H}_{37}}{\underset{\text{OH}}{\text{C}}}\text{C}_{17}\text{H}_{35}$ , (m. p. 58-59°). *n*-Octadecyl-

magnesium chloride was synthesized in 64 per cent yield from *n*-octadecyl chloride. Apparently, there was much less coupling product present (*n*-pentatriacontane) than with *n*-octadecylmagnesium bromide. 18-*n*-Octadecylpentatriacontan-18-ol was obtained in 86 per cent yield from stearone and *n*-octadecylmagnesium chloride.

18-*n*-Octadecylpentatriacontan-18-ol yielded 18-iodo-18-*n*-octadecylpentatriacontane (m. p. 29-32°) on treatment with phosphorus and iodine. An attempt to couple the iodide in a Wurtz reaction gave an unsaturated mixture (m. p. 40-42°). It was considered to be the disproportionation products, 18-octadecylpentatriacontane and 18-octadecylpentatriacontene. Dehydration of 18-*n*-octadecylpentatriacontan-18-ol with *p*-toluenesulfonic acid yielded 18-*n*-octadecylpentatriacontenes (m. p. 42-44°), which was possibly a mixture of olefins. 18-*n*-Octadecylpentatriacontane (m. p. 45-46°) was obtained by the reduction of 18-iodo-18-*n*-octadecylpentatriacontane with zinc and hydrogen chloride in acetic acid.

No differences were observed between the preparation and reactions of the compounds mentioned above, in comparison with other members of a shorter chain normal aliphatic series.

# BREAKDOWN POTENTIALS OF GASES UNDER ALTERNATING VOLTAGES

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Received July 19, 1939

This investigation was devoted to a study of the starting potentials of gases of commercial purity for frequencies less than one million cycles per second. The gases used were hydrogen, nitrogen, helium and argon. The discharge tube was of spherical design having spherical aluminum electrodes three-fourths inch in diameter. Five gap distances were used ranging from 10 m.m. to 50 m.m.

Two methods were used to generate the A. C. voltages. The first consisted of amplifying standard frequency signals received from a primary frequency standard available in the laboratory. This method was used to generate voltages of one thousand, ten thousand, and one hundred thousand cycles per second.

Because the amount of one megacycle energy available from the frequency standard was very small it was decided to generate the necessary voltage with a local oscillator tuned to synchronism with the standard. This oscillator consisted of a WE211E vacuum tube in a standard tuned-plate-tuned-grid circuit.

The method of measuring the excitation voltage consisted of rectifying the voltage and charging a condenser to its peak value. The voltage across the condenser was easily read with a high resistance direct current voltmeter. Half wave rectification was used on all frequencies up to one hundred thousand cycles. The meter under these conditions read the peak value of the voltage applied to the discharge tube. In order to avoid capacity effects to ground at one megacycle full wave rectification was used. The meter under this condition read one-half the voltage applied to the discharge tube.

A sample of the gas was placed in the system to a pressure that would give a starting potential of the order of 1000 volts. The pressure was then reduced by small intervals and a voltage reading taken at each pressure. This procedure was continued until the pressure was so low that the 1000 volts available was not sufficient to start the discharge. The starting potential was obtained by slowly increasing the potential and observing the voltage at the point of breakdown. A second sample was then admitted and the above procedure repeated. It was originally planned to repeat this procedure a third time but it was found that the points obtained from the second sample were in such good agreement with those of the first that it was decided to omit the third run. This procedure was repeated for the five gap distances.

The above procedure was repeated for each of the four gases hydrogen, nitrogen, helium and argon; and for each of the four frequencies one thousand, ten thousand, one hundred thousand, and one million cycles per second.

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<sup>1</sup> Original thesis submitted July, 1938. Doctoral thesis number 484.



The curves of the starting potential versus the pressure show that there is a definite tendency for the usual straight line portion to be concave downward. J. Thomson's theory (1) results in the following expression for plane parallel electrodes

$$V_s = Ax + \frac{B}{x}$$

where  $x$  is a number proportional to the mean free path of an electron. By multiplying this expression by  $x^{-3}$  one obtains an expression that will yield a curve similar to those obtained. It was sought, without avail, for a justification of this term. It is believed that the type of electrodes used might explain this effect.

Curves of the starting potential versus the logarithm of the frequency were plotted. It appears that there is a definite decrease in the starting potential for frequencies of approximately one million cycles per second. An explanation for this was not obtained although a theory based on the assumption that an electron makes a large number of collisions throughout its travels for one-half cycle gives a fair check on the order of magnitude.

Curves of the logarithm of the pressure versus the logarithm of the gap distance for each gas at each of the four frequencies were plotted. This method of representing the data was suggested by Slepian (2). On this basis one obtains parallel lines provided the starting potential is a function of the product of the pressure and the electrode separation. For gap distances less than about 32 m.m. the curves rapidly approach parallel straight lines having the same slope. The trend for larger gap distances is definitely concave upward.

If Paschen's law holds for plane parallel electrodes only, one would expect to obtain parallel lines of slope equal to  $-1$ .

Recognizing the fact that the field strength produced by spherical electrodes, averaged throughout the path of a particular line of force, is, in general, less for a given electrode separation than it would be for plane parallel electrodes, one would expect the starting potential to be larger for the spherical electrodes. This statement is true provided an electron, in one-half cycle, travels a distance comparable to the dimensions of the tube. This explains the concave upward property of the curves and suggests that, as the gap distance becomes smaller, the conditions of the discharge approach those of the plane parallel electrodes. The curves all approach the same slope for small gap distances, thus suggesting that the same relations hold for all the gases at all the frequencies used.

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# IONIZATION CONSTANTS AND INSECTICIDAL ACTION OF SUBSTITUTED QUINOLINES AND TETRAHYDROQUINOLINES<sup>1</sup>

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Received July 19, 1939

A systematic study was made on the relationship between structure and toxicity to insects of a series of alpha-substituted quinolines and tetrahydroquinolines.

The quinolines were synthesized by treating quinoline or quinaldine with organometallic compounds such as  $\text{RMgX}$  and  $\text{RM}$ . Reduction to the corresponding tetrahydroquinolines was achieved by means of metallic sodium in boiling ethyl alcohol.

The ionization constants of the reduced bases were determined in absolute methyl alcohol using the hydrogen electrode in a manner analogous to that used by Goodhue<sup>2</sup>. The constants found showed that the electron-sharing ability functions developed by Hixon and Johns<sup>3</sup> and co-workers<sup>2,4</sup> can be extended to include the quinoline nucleus.

For the toxicity work the common firebrat, *Thermobia domestica* (Pack.), was chosen. The apparatus of Tattersfield as modified by Craig and Richardson<sup>5</sup> was used. For the spraying chamber a bell jar was arranged fitted with an atomizer located in the top and centered to direct its spray into a 10 cm. crystallizing dish containing the test insects. Samples of ten insects were sprayed with varying concentrations of the bases dissolved in a 75 per cent acetone-water mixture. The use of acetone eliminated the use of a spreader and controls run on this solvent showed no mortalities.

Stock solutions of 4 per cent, expressed as grams per 100 cc., were made up and these were diluted for the lower concentrations. Near the center of the mortality curves five or six samples were sprayed while the extremities of the curve were located by spraying in triplicate or duplicate. Mortality counts were made at the end of 48 hours and all paralyzed insects were counted as dead. The concentrations giving 50 per cent mortality were determined by drawing eye-fitted curves through the data plotted on log-probability paper.

The substituted tetrahydroquinolines were more toxic than the corresponding quinolines in every case except one. The one exception was  $\alpha$ -n-butylquinoline and this compound was the most toxic one tested, either reduced or unreduced. No explanation was apparent for this and the unusual toxicity did not extend further along the series to the next member,  $\alpha$ -n-amyloquinoline.

No simple relation existed between the ionization constants and the toxicities of the tetrahydroquinolines. The deviations could not be ex-

<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 527.

<sup>2</sup> Goodhue and Hixon, *J. Am. Chem. Soc.*, **56**:1329 (1934).

<sup>3</sup> Hixon and Johns, *ibid.*, **49**:1786 (1927).

<sup>4</sup> Starr, Bulbrook, and Hixon, *J. Am. Chem. Soc.*, **54**:3971 (1932)

<sup>5</sup> Craig and Richardson, *Iowa State Coll. J. Sci.*, **7**:477 (1933).

plained by the variances in the physical properties of the bases and it appeared that a combination of factors was responsible for the results obtained.

The results are summarized in table 1 for the tetrahydroquinolines. The concentrations giving 50 per cent mortality are compared with the ionization constants as well as with the boiling points of the free bases.

TABLE 1. *Comparison of toxicities to Thermobia domestica of some alpha-substituted tetrahydroquinolines with their physical constants*

| Tetrahydro-<br>quinolines | Approximate concentration<br>giving 50 per cent mortality |                        | Ionization<br>constant<br>in methanol | Boiling<br>point             |
|---------------------------|---|------------------------|---------------------------------------|------------------------------|
|                           | gm./100 cc.<br>of solution                                | Molar<br>concentration |                                       |                              |
| $\alpha$ -n-butyl .....   | 1.3   | .069                   | $.813 \times 10^{-11}$                | 138°/6 mm.                   |
| $\alpha$ -ethyl .....     | 1.2   | .075                   | $1.31 \times 10^{-11}$                | 110-3°/5 mm.                 |
| $\alpha$ -methyl .....    | 1.5   | .102                   | $1.22 \times 10^{-11}$                | 125°/17 mm.                  |
| $\alpha$ -H .....         | 1.15  | .087                   | $1.23 \times 10^{-11}$                | 126°/18 mm.                  |
| $\alpha$ -p-tolyl .....   | 2.30  | .103                   | $1.11 \times 10^{-12}$                | 210-2°/14 mm.                |
| $\alpha$ -phenyl .....    | 1.85  | .089                   | $.653 \times 10^{-12}$                | 196°/8 mm.                   |
| $\alpha$ -o-tolyl .....   | 1.90  | .085                   | $.645 \times 10^{-12}$                | 200-2°/6 mm.<br>(m. p. -69°) |
| $\alpha$ -mesityl .....   | 1.90  | .076                   | $1.20 \times 10^{-12}$                | 218°/6 mm.                   |



# FINITE STRAIN ANALYSIS IN ELASTIC THEORY<sup>1</sup>

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In the elementary mathematical theory of elasticity only those terms are retained in the expressions for the unit strains which are linear in the space derivatives of the displacement vector. When terms involving squares or products of the derivatives are also retained the strains are termed "finite". The author treats the stability of the clamped thin circular plate with edge loading and the bending of the clamped moderately thick circular plate under normal loading for the case of finite strains.

The stability problem for the clamped circular plate begins with the decomposition of the v.Karman (1) non-linear differential equations into two systems of ordinary differential equations by means of parameter expansions of the slope and radial membrane stress functions. This constitutes an approximation method for solving the non-linear problem. The first set of equations yields the elementary solution of the problem. The edge loading for the higher approximations is found to depend on a result due to Mason (2) on the exceptional case in characteristic boundary value problems. On the second approximation the membrane stress is found to decrease at the center and increase at the edge of the plate while the slope function is determined up to two indefinite integrals which remain unintegrated. The indeterminacy resulting in the exceptional case is to be removed by minimizing the problem when put in the variational form.

A method of Garabedian (3) is used to derive the differential equation of bending for the moderately thick circular plate with finite strains. A parameter expansion of the displacements results in sequences of equilibrium equations and surface traction conditions which are ordered in the parameter. If the equilibrium equations are integrated one by one and the corresponding surface traction conditions applied and this repeated a sufficient number of times, it is possible to obtain a non-linear differential equation for the first order term in the deflection of the middle surface of the plate. Repetition of this process yields the differential equation for the second term, etc.

The first differential equation is solved for the clamped plate. It is found that the loading must be of the order of  $10^2$  as compared to the order of  $10^1$  in the case of the thin plate before any significant deviation from the elementary solution for the deflection is noted. The distinction between the two cases is due to the fact that the v.Karman equations for the thin plate, although the vertical shear is neglected, take account of the lifting effect of the membrane stresses. On the other hand the equations for the moderately thick plate do not include the membrane stress effect and the deviation from the elementary solution is due entirely to the non-linear character of the strains when expressed in terms of the derivatives of the displacements.

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<sup>1</sup> Original thesis submitted June, 1939. Doctoral thesis number 517.

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## SOME STUDIES ON SWINE INFLUENZA<sup>1</sup>

### I. COMPARATIVE STUDY OF *HEMOPHILUS INFLUENZAE* SUIS AND *HEMOPHILUS INFLUENZAE*

### II. ANTIBODY RESPONSE TO EXPERIMENTAL SWINE INFLUENZA

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Received July 19, 1939

### I. COMPARATIVE STUDY OF *HEMOPHILUS INFLUENZAE* SUIS AND *HEMOPHILUS INFLUENZAE*

The present report represents the second study of the relationships and differences existing between *Hemophilus influenzae suis* Lewis and Shope, 1931, and *Hemophilus influenzae* Pfeiffer, 1892. A previous comparative study by Lewis and Shope (1) had established a similarity between these two organisms on the basis of their morphology, growth and biologic characteristics, as well as cross-agglutination results with immune sera of relatively low agglutination titres. The present experiments were performed on 14 swine and 10 human strains of the organisms, using 12 carbohydrates and alcohols for fermentation studies and 8 rabbit immune sera for cross-agglutination and agglutinin-absorption studies. In addition, the production of indol and the cultural and morphologic characteristics of the organisms were studied as other possible means of differentiation.

The carbohydrate and alcohol fermentation results with the use of a basal medium containing a chocolate peptic-digest beef broth medium with Andrade's indicator established a distinct difference between the swine and human organisms. All of the swine strains fermented maltose and to a certain extent saccharose while the human strains failed to do so, with one exception. On the other hand, most of the human strains fermented xylose, dextrose, and galactose, which were not fermented by the swine organisms, with the exception of two strains, one of which fermented dextrose and the other galactose. One of the human strains studied behaved quite differently in that it actively fermented dextrose, maltose, saccharose, and galactose.

Indol was not produced by any of the swine strains, while 50 per cent of the human strains were indol positive, which agrees with the usual percentage figures given in the literature. No other physiologic, cultural or morphologic differences of importance were observed between the two organisms.

Serologically, the differences between the human and swine organisms were not as clear-cut as those of the biochemic results, but the cross-agglutination results gave sufficient qualitative, and in some cases, quantitative titre differences, to allow the segregation of these organisms into separate types. Wherever these differences were not sufficiently marked, the cross-agglutination results on the homologously absorbed sera estab-

<sup>1</sup> Original thesis submitted October, 1938. Doctoral thesis number 491.



lished these agglutination titres as mainly of a secondary antigenic nature, demonstrating thereby the basic differentiation of the swine organisms, as a group, from the human organisms. Still, the cross-agglutination titres occurring between the human and swine strains proved that the two organisms were rather closely related.

By the cross-agglutination study of the homologously absorbed sera, the swine organisms were shown to form two subgroups, but the significance of this finding could not be determined since it could not be correlated to any other of the characteristics studied.

A distinct individuality in serologic behavior could be observed between all of the organisms but these differences were considerably smaller within the strains of swine origin. This relatively close relationship of the swine strains was attributed to the high degree of hyperimmunization of the rabbits providing the immune sera, which probably caused a loss in specificity due to secondary antigen effects.

## II. THE ANTIBODY RESPONSE OF SWINE TO EXPERIMENTAL SWINE INFLUENZA

Following the work of Andrews, Laidlaw and Smith (2) and Shope (3) who were able to transmit the human and swine influenza viruses to mice, this animal has become the most important means of studying the immunity response of both human and swine influenza. In the present study, the mouse was used routinely in the determination of the presence or concentration of neutralizing antibodies in the blood-serum of swine infected with the swine influenza virus or with the swine influenza virus and the *Hemophilus influenzae suis* organism. Previous to this time several individual and often incomplete reports had been given on the "neutralizing-antibody" response to human influenza. In this disease, antibodies had been shown to make their first appearance sometime after the third or by the seventh day, reaching their highest levels by the fourteenth or twenty-first days, and showing a decrease by the forty-fourth to the eighty-first days, if the maximum titres were high. The average values of the antibody titres of human patients at the beginning, or acute stage, of the disease were 1:21, those at convalescence were 1:305, while five months after infection the titres were 1:162.

The results of the successive neutralizing-antibody titrations with swine sera from animals infected with experimental swine influenza or the "filtrate disease" indicated that these antibodies made their first appearance at, more or less, the seventh day after infection. The titres increased rapidly after that time, reaching their maximum values at the fourteenth to the twenty-seventh days, showing a tendency to decrease by the eighty-fourth day, in one of the sera tested. The swine which developed an attack of typical swine influenza, with temperatures of 40° to 41.6° C. and a severe cough and labored respiration, showed a disappearance of these symptoms coinciding rather closely with the appearance of antibodies.

The two animals with the milder "filtrate-type of disease", with the absence of temperature and the presence of only the mildest symptoms for the first two days of the infection, showed the antibodies making their first appearance sometime between the seventh and tenth day. Maximum titres were not attained until the twenty-seventh day, which was some-

what later than for the animals having had a typical attack of swine influenza.

The time required for these antibodies to reach maximum levels and the height of these titres were otherwise quite individual with the six animals tested in this experiment. The average antibody titre for the five animals tested for more than eleven days was 1:108.

In comparison to the human influenza literature, the antibody response of swine to the disease provided an additional factor establishing the similarity of the two diseases and of their etiologic agents.

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# RELATION BETWEEN THE FREE ENERGY OF SOIL WATER AND THE MOISTURE CONTENT OF THE SOIL<sup>1</sup>

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Received July 19, 1939

During recent years it has become increasingly clear that the security with which water is held by soil can be measured and expressed in energy units. Such measurements are useful in characterizing soil moisture and are applicable to dynamic as well as static moisture systems. The moisture-energy relations of four Iowa soils ranging in texture from a fine sand to a silty clay have been investigated from saturation to oven dryness.

Porous ceramic cells were used for studying the sorption curves below pF 3.0. Soils of known volume weight were placed in double-walled porous pots and the equilibrium soil moisture contents at various soil moisture tensions were determined. Hysteresis effects caused the sorption curves to be displaced somewhat from the desorption curves.

Desorption curves were obtained for the Marshall silt loam and the Clarion sandy loam by plotting the moisture content of soil samples taken next to field tensiometers against the tensiometer readings. It was found that the sorption curves for Marshall silt loam are not significantly different at the six and twelve-inch depths. Similar curves for the Clarion sandy loam show that the coarse texture at the twelve-inch depth causes the moisture sorption curve for that depth to lie in a lower moisture range than the curve obtained from the six-inch installations.

Equilibrium moisture contents at pF values below 1.2 were obtained by placing soil samples on quartz sand columns that were in contact with a water table at a known distance below the soil sample. The lower end of the desorption curve can be used in characterizing the equivalent pore-size distribution in undisturbed soil samples since the shape of the curve is related to the proportion of pores that are emptied as the pF increases.

A centrifuge technique was developed for studying the desorption curve between pF 3.0 and 4.2. A 5 mm. soil column was placed on the upper end of an inverted porous cup that was in contact with a water table at a known distance below the soil sample in a centrifugal field. The relation between the capillary potential,  $\psi$ , and the centrifugal field force,  $rw^2$ ,

is given by the equation  $\frac{d\psi}{dr} = rw^2$  in which  $r$  is the radial distance

from the axis of rotation and  $w$  is the angular acceleration. Since at the water table  $r = r_1$  and  $\psi = \psi_1 = 0$ , the value of  $\psi$  at the center of the soil

column where  $r = r_2$  is  $\psi_2 = \frac{w^2}{2} (r_2^2 - r_1^2)$ . The desorption moisture

content of each of the four soils was obtained at several values of  $\psi$  be-

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<sup>1</sup>Original thesis submitted June, 1939. Doctoral thesis number 511.



tween pF 2.85 and 4.25 by using different values for  $r_1$ ,  $r_2$  and  $w$ . At pF 3.33 soil samples reached equilibrium moisture content after two hours of centrifugation whereas eight to ten hours were necessary at pF 3.98. The rate of approach to moisture equilibrium can probably be increased by changing the design of the inverted porous cup.

The moisture sorption curves were established by a vapor pressure technique over the range of moisture contents between oven dryness and the wilting point. Soil samples were allowed to reach a moisture equilibrium in evacuated constant temperature desiccators containing sulfuric acid solutions of known concentrations. The pF of the soil water was calculated from the aqueous vapor pressure of the acid solutions. A hysteresis effect that has been observed at lower pF values extends to the drier portions of the sorption curves and amounts to ten to fifteen per cent of the desorption moisture content between pF 4.5 and 6.0. By extrapolating the desorption curves it was found that they intersect the pF axis at  $7.00 \pm 0.02$  which corresponds to a relative humidity of 0.081 per cent at  $25^\circ \text{C}$ . and to a relative humidity of 0.40 per cent at  $110^\circ \text{C}$ .

Data obtained by the different experimental techniques were found to be in close agreement and were summarized in the form of complete desorption curves for each of the soils. The relations between the moisture content and the pF is expressed by the following empirical equations.

|                           |   |
|---------------------------|---|
| Dickinson fine sand ..... | $\text{pF} = 7.0 - 0.87\theta + 0.042\theta^2$  |
| Clarion sandy loam .....  | $\text{pF} = 7.0 - 0.44\theta + 0.010\theta^2$  |
| Marshall silt loam .....  | $\text{pF} = 7.0 - 0.26\theta + 0.0032\theta^2$ |
| Wabash silty clay .....   | $\text{pF} = 7.0 - 0.17\theta + 0.0012\theta^2$ |

It was found that the desorption equations for the individual soils could be derived from the following generalized equation

$$\text{pF} = 7.0 - \frac{3.3\theta}{\text{P.W.P.}} + \frac{0.53\theta^2}{(\text{P.W.P.})^2}$$

The pF at the permanent wilting percentage of the four soils studied decreases as the soil texture becomes finer and may be expressed by the equation.

$$\text{pF}|_{\text{P.W.P.}} = 4.40 - 0.02 \text{ P.W.P.}$$

Although it does not represent an equilibrium moisture content the hygroscopic coefficient was found to correspond to an approximately constant pF. For the four soils studied the pF at the hygroscopic coefficient was  $4.54 \pm 0.10$ .

By assuming that the outer soil boundary of a moisture equivalent sample is saturated, it was calculated that the average value of the capillary potential in a standard moisture equivalent sample should be  $4.91 \times 10^5$  ergs/gm. which corresponds to a pF of 2.70. The experimental value of the pF at the moisture equivalent was found to range from 2.55 to 2.81 with an average value of 2.66. This corresponds to a tension of 34 cm. of mercury and is in agreement with previous determinations at Iowa State College and with the calculated value of 2.70.

# THE RELATION BETWEEN METHODS OF SACCHARIFICATION AND YIELD OF ETHANOL FROM VARIOUS CEREALS<sup>1</sup>

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Received July 19, 1939

The chemical approach to problems of fermentation is a comparatively recent development in a process the beginnings of which are obscured in the mists of antiquity. Undoubtedly primitive man knew that, on standing, fruit juices were converted into intoxicating liquors, but it was not until the last century that the fermentation of sugars by yeast began to receive scientific attention. Early interest in the process was due solely to the beverages formed, but more recently there has developed a large demand for industrial alcohol, which is produced chiefly from blackstrap molasses or cereal grains.

In the production of ethanol from starchy grain mashes, the starch first must be converted to fermentable sugars. This process of hydrolysis may be accomplished through the use of the enzyme, amylase, or by heating the starch with dilute acid. In commercial practice, the enzyme contained in barley malt is employed to effect this saccharification. Malt, however, is relatively expensive, and a more economical process would be very desirable. Accordingly, the material presented in this thesis deals with the relation between methods of saccharification and the yields of ethanol from corn mashes. Saccharification by the amylases of malt, soybeans, fungi and bacteria was investigated, and the study included the effects of combinations of these various amylolytic materials on normal, thick, and acid-hydrolyzed mashes.

## METHODS

Fermentation mashes were prepared by cooking mixtures containing corn meal and water in the proportion of 20 g. of corn meal to 100 ml. of distilled water. The three types of mashes studied were, (1) normal mashes thinned slightly by cooking with a small amount of acid or by "pre-malting"; (2) thick mashes prepared by cooking with a small volume of dilute acid and then diluted with distilled water to fermentable concentrations; and (3) acid-saccharified mashes prepared by cooking with dilute acid until about 90 per cent hydrolysis of starch to dextrose was effected. These mashes, except the "pre-malted" ones which needed no adjustment, were adjusted to pH 5.0 with ammonium hydroxide and were saccharified at 55° C for sixty minutes by the addition of various amylolytic materials. After cooling, the mashes were inoculated with an actively growing yeast culture in 10 per cent beer wort and fermented for three days at 30° C. The yeast employed was a pure strain of *Saccharomyces cerevisiae*. The fermented mashes were distilled and the ethanol was determined from the specific gravity of the distillates. After correcting for the ethanol derived from the inoculum and the amylolytic materials, the yields were calculated from the following theoretical equation:

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<sup>1</sup> Original thesis submitted June, 1938. Doctoral thesis number 515.

$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$ . The dextrose equivalent of the starch was determined by the official A. O. A. C. direct acid hydrolysis method combined with the Shaffer-Hartmann method of dextrose determination.

The molds and bacteria were cultured, in Erlenmeyer flasks, on media prepared by adding water to wheat bran, distillery residues, corn gluten meal or corn meal, and the fermented media were employed directly as amylolytic materials. The molds were cultivated in larger quantities on the most suitable substrate, moistened wheat bran, in a revolving drum. After growth for about two days, the resulting moldy brans were air-dried at room temperature, ground and stored for future use as amylolytic agents. Likewise, the soybeans and barley malt were ground before use.

## EXPERIMENTAL RESULTS

### A. Amylase Production by Various Microorganisms

Amylase production by 17 species or strains of molds and by 7 species of bacteria was investigated by culturing the organisms on suitable substrates and then by using these materials to saccharify corn mash. The mash was prepared by mixing 60 g. of corn meal, 0.6 g. malt and 300 ml. hot water in 300-ml. Erlenmeyer flasks, the malt serving to reduce slightly the viscosity of the mash. The mixtures were cooked for thirty minutes at 20 pounds steam pressure and saccharified according to the procedure described above. The ethanol obtained from the fermentation of these mash served as a quantitative measurement of the saccharifying powers of the various amylolytic materials. None of the fermentations using bacterial preparations gave satisfactory ethanol yields but, when cultivated on moist wheat bran, a number of the molds yielded as much as or more alcohol than did malt. A strain of *Aspergillus oryzae* (Laboratory No. 2; American Type Culture Collection No. 4184) gave the best results, and the moldy brans prepared in a revolving drum using this organism were employed in further investigations.

### B. Effect of Amylolytic Materials on Acid-thinned Normal Mash

Acid-thinned normal mash was prepared by cooking mixtures of 100 g. of corn meal and 500 ml. of 0.02 normal hydrochloric acid in 1-liter Erlenmeyer flasks for thirty minutes at 20 pounds steam pressure and neutralizing to pH 5.0 with ammonium hydroxide. The saccharification and fermentation were accomplished in the manner described above, using malt, moldy bran and soybean meal as saccharifying agents. The soybean meal was relatively ineffective, but ethanol yields up to 87 per cent were obtained using moldy bran as compared to about 72 per cent using malt. Binary combinations of malt, moldy bran and soybean meal resulted in slightly higher yields than were obtained from the individual substances used alone. The malt-soybean meal combinations were not as efficient, however, as moldy bran, either singly or in combination.

### C. Effect of Amylolytic Materials on Acid-saccharified Mash

Acid-saccharified mash was prepared by cooking mixtures of 100 g. of corn meal and 500 ml. of 0.10 normal hydrochloric acid in 1-liter Erlenmeyer flasks for two and one-half hours at 25 pounds steam pressure and neutralizing to pH 5.0 with ammonium hydroxide. These mash



were fermented as before, both with and without addition of amylolytic materials. Direct fermentation of acid-saccharified mashers gave ethanol yields of about 75 per cent which were much lower than those from normal mashers saccharified by moldy bran. The addition of moldy bran to acid hydrolysates caused a marked increase in ethanol yields (up to 92 per cent). Malt and soybean meal in low concentrations also increased the ethanol yields but to lesser degrees than did moldy bran. The synergistic effect of combinations of soybean meal with malt or moldy bran were no more effective than was moldy bran alone. The low yields from the direct fermentation of acid hydrolysates were shown to be due to incomplete saccharification of the starch rather than to a lack of yeast growth stimulants or to the presence of toxic materials.

#### *D. Effect of Amylolytic Materials on Thick Mashers*

Thick mashers were prepared by cooking mixtures of 100 g. of corn meal and 167 ml. of 0.12 normal hydrochloric acid for thirty minutes at 20 pounds steam pressure. The hot mashers then were diluted with 333 ml. of warm distilled water. This procedure was developed in order to lessen the cost of cooking if employed in large scale production. The pH was adjusted, and the mashers were saccharified and fermented in the customary manner. Using the same amylolytic materials, singly and in combinations, the fermentation results were essentially the same as those from normal mashers except that only about 80 per cent as much amylolytic material was required for thick mashers.

# THE CULTURE OF FLY LARVAE FOR USE IN MAGGOT THERAPY<sup>1</sup>

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Received July 19, 1939

The object of this investigation<sup>2</sup> was to devise efficient and economical methods for the culture of brood larvae, brood flies, sterile larvae, and for the subsequent retardation and transportation of the sterile specimens. *Lucilia sericata* Meigen was the species of fly used, and tests were conducted at a temperature of 26.5° C. unless otherwise stated.

## CULTURE OF BROOD LARVAE

Brood larvae are those reared for maintaining the progeny, and large healthy specimens are essential for maximum egg production by the subsequent flies.

Employing 10 eggs per gram of beefsteak, and using 150 grams per test the subsequent maggots averaged 46.5 milligrams each, in weight. Specimens reared on beef heart averaged 46.3 milligrams, and those reared on beef brain averaged 42.2 milligrams each. Because of the slightly larger size of specimens reared on beefsteak, and because it is one of the most readily available of meats, its use is preferred.

## CULTURE OF BROOD FLIES

A food modified from Baer<sup>3</sup>, and consisting of water 70 parts, extracted honey 30 parts, one egg, and one-fourth cake of Fleischmann's yeast per 200 cc. of solution was found, under controlled conditions, to maintain flies to an average age of 43.8 days. The average maximum egg laying period per cage of 12 female flies was 60.3 days, with an average production of 55.4 eggs per day per female. Flies fed sugar, banana, or milk and honey produced less than one third as many eggs, and were shorter lived. The eggs laid by flies fed banana, or milk and honey were, furthermore, exceedingly low in viability.

## CULTURE OF STERILE LARVAE

*Separation of the eggs:* Eggs are laid in clusters and must be separated prior to sterilization. A rapid and efficient method consists of a brief softening of the clusters between pieces of wet black cloth, and then separating by spreading thinly with a flexible blade spatula.

*Disinfection of eggs:* After separation, eggs are sterilized by immersion in test tubes of the disinfectant. Experiments were conducted with nine disinfecting solutions, as follows: formalin 5 per cent; formalin 5 per cent plus sodium hydroxide 1 per cent; formalin 10 per cent plus sod-

<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 494.

<sup>2</sup> The data presented in this paper were secured from research carried on by the author as an employee of and under a project authorized by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

<sup>3</sup> Baer, W. S., Jour. Bone Joint Surg., 13: 438-475 (1931).

ium hydroxide 1 per cent; formalin 5 per cent plus potassium hydroxide 1 per cent; formalin 5 per cent plus Labarraque's solution 2 per cent; saturated solution of calcium hypochloride; mercuric chloride 1:2000, alcohol 25 per cent, and hydrochloric acid 0.5 per cent; phenol 2 per cent; and sodium hydroxide 4 per cent for three hours, followed by formalin 5 per cent for 30 minutes.

After testing, the solutions were rated relative to their disinfecting values on eggs, effect on egg viability, egg agglutination, floating, time required for sterilization; and adaptability for use on freshly laid eggs. A combination of formalin 5 per cent plus sodium hydroxide 1 per cent, when used for five minutes gave the most satisfactory results. A 77.6 per cent hatch was obtained from eggs sterilized with this solution, and sterility was 100 per cent in all tests conducted. Unlike most of the disinfectants tried this one did not cause separated eggs to agglutinate or float, and could be used on freshly laid eggs without seriously affecting their viability.

#### RETARDATION AND TRANSPORTATION OF STERILE MAGGOTS

(a) *Retardation by low temperature:* Retardation of surgical maggots enables them to be held for future use if not needed immediately, and permits their shipment to distant points. In cold storage surgical maggots fed as long as activity persisted, but very little feeding occurred at 4° C. At 10° to 11° C. 80.6 per cent fed, and at 20° to 21° C. 98.5 per cent fed.

At a temperature of 10° to 11° C. maggots increased 23.4 per cent in weight during 72 hours. Specimens are frequently held for this period while awaiting results of the sterility tests, and growth beyond 23.4 per cent is intolerable. Twelve degrees C. was established, by test, as the maximum effective retardation-inducing temperature.

Mortality of surgical maggots in cold storage is high, reaching 63.3 per cent in 24 hours, and 78.6 per cent in 72 hours. It is evident that low temperature retardation is unsatisfactory.

To test the value of low temperature retardation of maggots in transit, specimens were packed on ice in commercially used cartons. A four point multiple thermocouple was placed at various points in the specimen bottle, and temperature readings made at half-hour or hourly intervals until air temperature was reached. Tests were conducted under shipping conditions of both summer (30° to 33° C.) and winter (20° C.) conditions.

Under summer conditions no effective retardation was accomplished as the temperature never dropped to the 12° C. level.

Under winter conditions an effective retarding temperature was reached in all portions of the maggot bottle, but for only 14 hours. Besides causing a great mortality, retardation did not last long enough for distance shipping. Low temperature retardation is therefore not effective in transportation of surgical maggots.

(b) *Retardation by nutritional insufficiency:* As low temperature retardation is unsatisfactory, tests were conducted to devise a method of retardation by nutritional means. A food consisting of evaporated milk one part, distilled water seven parts, and agar 1.5 per cent was found to retard both growth and development for a week or more without serious detriment to the maggots. After six days retardation 88.0 per cent of the specimens were in the second instar. As feeding in the wound takes place



mainly in the third instar it is seen that specimens would have four or five days of therapeutic value after retardation. Only 36 per cent of the maggots were in the third instar after 10 days of nutritional retardation.

Mortality with nutritional retardation is relatively low, being only 11.5 per cent after six days as compared to 87.0 per cent for specimens retarded by low temperature. From the third to the ninth day the mortality was 15.6 per cent with nutritional retardation, while with low temperature retardation all specimens were dead before the ninth day.

After three days of nutritional retardation 79.6 per cent of the specimens were able to resume feeding when placed on necrotic tissue, and removed 6.4 grams per unit of 100 newly hatched specimens. With low temperature retardation this is reduced to 21.4 per cent and 1.8 grams respectively. After six days nutritional retardation 70.0 per cent of the specimens resumed feeding, compared with only 5.1 per cent of those retarded by low temperature. Even on the ninth day 28.1 per cent of the nutritionally retarded specimens fed.

After three days of nutritional retardation 94.5 per cent of the specimens subsequently fed for four days on necrotic tissue. After six days retardation 75.4 per cent fed for four days, 39.0 per cent for five days, and 24.3 per cent for more than five days. Even after nine days retardation 29.1 per cent fed for four days, 39.5 per cent for five days, and 28.3 per cent for more than five days.

The value of nutritional retardation in transit was demonstrated by shipping specimens to various points in the United States, some as far as 3,000 miles. These specimens arrived in excellent condition and were able to subsequently feed for the required time in wounds.

Surgical maggots can be produced efficiently and economically by use of the proper technique in all steps of the breeding and disinfecting processes, and by the use of adequate methods for subsequent retardation.

# EFFECT OF QUARTZ FILTERS ON THE DISTRIBUTION OF ENERGY IN LAUE PATTERNS<sup>1</sup>

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Received July 19, 1939

It has been shown by Fox and Carr (1) that the Laue pattern from Y-cut quartz plates oscillating piezoelectrically in the fundamental mode is more intense than the pattern from the non-oscillating plates. Nishikawa and others (4) have observed the same effect with X-, Y-, and R-cut plates oscillating at the fundamental and third harmonics. In the third harmonic mode, they observed four spots instead of the usual two. Klauer (3) has observed the same effect when he changed the angle between the primary beam and the face of the crystal plate by as much as 72 degrees. Fox and Fraser (2) reported an increase of about twenty per cent in the central beam during oscillation.

No quantitative measurements of the amount of energy of a definite wavelength extracted by a given crystal have ever been made. Nor is it agreed that the intensity of the central beam is increased with oscillation.

The purpose of this research was: (1) to determine if all the energy of a definite wavelength is extracted by a crystal; (2) to study the effect of oscillation on the intensity of the central beam; (3) to study the effect of oscillation on the intensity of Laue spots produced by quartz-filtered radiation.

## METHOD OF PROCEDURE

For all phases of this investigation, the same set-up was used. It involved a crystal holder which could support two crystals in the same orientation. Between the crystals was a sandwich composed of a lead plate between two brass plates. A hole through the sandwich, concentric with those in the collimator, served to absorb the Laue pattern of the first crystal and transmit only the central beam. Both AT- and Y-cut quartz plates were used.

## RESULTS AND DISCUSSION

If all the energy of the critical wavelengths is diffracted to the Laue spots, then the central beam should be deficient in such wavelengths in which case a second crystal of identical orientation should give no diffraction pattern when placed in the central beam from another crystal. In the case of both AT- and Y-cut crystals good Laue patterns of the second crystal were obtained. Thus, a crystal does not extract all the energy of a given wavelength from a central beam.

If the intensity of the central beam is increased with oscillation, then a Laue pattern formed with the central beam from an oscillating crystal should be more intense than one formed with the central beam from the non-oscillating crystal. An investigation of this idea was made using AT-

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<sup>1</sup> Original thesis submitted July, 1938. Doctoral thesis number 488.

cuts oscillating at the fundamental and third harmonics and Y-cuts oscillating at the fundamental. In no case was any difference in intensity observed.

If an appreciable portion of the energy of the critical wavelength is absorbed in passing through one crystal, then it is conceivable that oscillation of a second crystal would not produce so great an effect on the intensity of its pattern formed with the energy from the central beam of the first crystal. AT-cut plates were oscillated at the first and third harmonics and Y-cut plates at the fundamental, and in each case an increase comparable to that found in the oscillation of a single crystal was observed.

When both crystals were oscillated, the increase in intensity was slightly greater than that observed when only the second crystal was oscillated. This appears to be an "anomalous" result.

The energy which is diffracted to a Laue spot comes from the small crystallites on the surfaces of the crystal. All the energy which is diffracted by the interior of the crystal suffers extinction due to the ordered array of that region. Because an individual crystallite is not thick enough to extract all the energy of its particular critical wavelength, there is still a considerable portion of that wavelength remaining in the central beam, and consequently a second crystal will show a good Laue pattern when placed in the beam.

During oscillation, the interior of the crystal becomes deformed so that the angle between the central beam and the crystal planes is altered. Therefore, some energy of wavelengths adjacent to the critical wavelength will be diffracted and the critical wavelength will not suffer extinction. However, the range of wavelengths diffracted by the deformed interior of the crystal is small compared to the range diffracted by the crystallites so that no change in the intensity of the second crystal would be expected.

Now the central beam of a crystal will contain wavelengths which can be diffracted by the deformed portion of an oscillating crystal so one would expect to get an increase in intensity of the Laue pattern when the second crystal is oscillated. This effect was observed.

When both crystals are oscillating, the following conditions are obtained. The central beam from the first crystal contains some of the critical wavelength for the non-oscillating crystal. This is diffracted by the second crystal and increases the intensity. Thus, in this case, we have the intensity that we would normally get when the second crystal alone is oscillating plus the intensity caused by the energy of the critical wavelength which is now incident on the second crystal. It might be argued that under these conditions, an increase of intensity should be observed when the first crystal is oscillated. However, in the latter case, the critical wavelength suffers extinction in the second crystal.

#### SUMMARY AND CONCLUSIONS

A double crystal set-up with a collimator between the two crystals was used to determine whether (1) a crystal extracts all the energy of a given wavelength; (2) the intensity of the central beam of a Laue pattern is increased when the crystal oscillates.

It is impossible to say from this study whether a crystal extracts all the energy of a given wavelength. Oscillation of a crystal does not change



the intensity of the central beam although it does alter its composition.

The author wishes to express his appreciation to Professor G. W. Fox for his encouragement and many suggestions in this work, and to Professor J. W. Woodrow for making it possible to obtain adequate equipment to carry on the research.

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# SOME FACTORS INFLUENCING NITROGEN FIXATION BY AZOTOBACTER<sup>1</sup>

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Received July 19, 1939

## DISTRIBUTION OF AZOTOBACTER IN FIELD SOILS

The purpose of this part of the investigation was to determine the influence of certain fertilizer treatments on the distribution of Azotobacter and on the nitrogen fixing power of soils from various experimental fields scattered throughout the state and located on the major soil types. Three composite soil samples were taken from each experimental field, one from the check plot, one from the plot which had been treated with manure and lime and a third from a plot which had been treated with manure, lime and superphosphate. The presence of Azotobacter was sought by means of Winogradsky's spontaneous culture test (starch as energy source) and by the use of silica gel plates (mannitol as energy source). In addition the amounts of nitrogen fixed on the silica gel plates and in solution culture were determined along with the pH, lime requirement, and amount of water-soluble phosphorus in the soil.

Soil reaction is one of the important factors affecting the occurrence of Azotobacter in these soils. Different results were secured, however, with the two different methods used to detect the presence of these organisms. Many investigators have reported that Azotobacter do not occur in soils more acid than pH 6.0. In the work reported only four per cent of the soils more acid than pH 6.0 were positive according to the spontaneous method, but 64.5 per cent of the soils more acid than pH 6.0 contained the organisms according to the silica gel method. The silica gel method is probably more reliable for testing the presence of Azotobacter in soils than the spontaneous method. However, within that group of soils which were positive with the silica gel method the more nearly alkaline soils usually had the highest nitrogen fixing power, indicating that they either contained more organisms or those present were more active.

The fertilizer treatments which the soils had received in the field increased the percentage of soils containing the organisms. Sixteen per cent of the unfertilized soils contained Azotobacter according to the spontaneous test, but 64 per cent of the fertilized soils showed the presence of these organisms. The treatment of acid soils, many of them apparently Azotobacter free, with enough lime and manure to bring the reaction to near pH 6.0 resulted in the establishment of an Azotobacter flora. The fertilized soils contained a greater quantity of water-soluble phosphorus than the untreated soils. The quantity of soluble phosphorus, however, appeared to have less influence on the occurrence of Azotobacter than the soil reaction.

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<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 496.

## ACCESSORY GROWTH FACTORS

Various materials likely to contain growth factors or to influence the environment were used in the culture media in which pure cultures of *Azotobacter* were grown. Of all the materials used soil extracts and powdered agar increased nitrogen fixation to the greatest extent. The stimulation obtained with the soil extracts could have been due to some of the rare elements such as vanadium, molybdenum or to some of the humic acids, as the soils with the highest content of organic matter increased the nitrogen fixation process to the greatest extent. No indication of any accessory factor essential to the growth of *Azotobacter* was obtained, nor of any factor markedly stimulating nitrogen fixation.

After the *Azotobacter* had been growing for many months in media made with pure chemicals, these organisms decreased in vigor and partially lost their power of utilizing atmospheric nitrogen. On the addition of brown sugar to the medium the organisms again grew more vigorously and fixed more nitrogen. There must be, therefore, some factor in the brown sugar which is necessary for maximum growth of these organisms. This stimulation could have been due to minerals, to nitrogen, or to accessory growth factors.

Hydrogen peroxide, methylene blue, yeast extracts, and vitamin B<sub>1</sub> decreased the quantity of nitrogen fixed. Cysteine hydrochloride, thioglycollic acid, inorganic sulphur compounds, inorganic nitrogen compounds, methyl phenyl acetate,  $\beta$ -indole 3 $\alpha$  propionic acid, extracts of grains, extracts of alfalfa and pasture grasses, manure, sand, and limestone did not have any significant influence on the quantity of nitrogen fixed.



SOME CHANGES PRODUCED IN GROWTH, REPRODUCTION,  
BLOOD AND URINE OF RATS BY SALTS OF ZINC WITH  
CERTAIN OBSERVATIONS ON THE EFFECTS OF  
CADMIUM AND BERYLLIUM SALTS<sup>1</sup>

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Received July 19, 1939

It was believed that an investigation of some of the changes produced by zinc salts might be of value in a study of the physiological and nutritional role of zinc. Zinc chloride, zinc sulfate and zinc carbonate were used in this investigation. Cadmium chloride, cadmium carbonate and beryllium sulfate were also used, and the results were compared with those obtained by means of zinc salts. The rat was chosen as the most desirable experimental animal for this investigation.

Young rats weighing thirty-five to forty-five grams were selected from the stock colony and were fed an adequate basal ration into which zinc carbonate was incorporated at levels of 0.10, 0.50 and one per cent zinc.

The one per cent zinc ration proved to be very toxic, and the rats lived only ten to twelve weeks. Food consumption on the one per cent zinc ration was considerably diminished; the rats became emaciated and showed anemia. The hemoglobin concentration and the number of red blood corpuscles were diminished in rats which received one per cent of zinc as zinc carbonate in the ration for five weeks.

The rats in the groups which received 0.50 per cent of zinc as zinc carbonate in the ration were studied for various lengths of time up to thirty-nine weeks. Some effects of the zinc feeding observed in the rats on this level included anemia, diminished food consumption, retardation in growth as measured by weight increase, loss of fertility in the female rats and greatly increased urine volume of the male rats. The anemic condition was evidenced by lowered hemoglobin values, but the number of red blood corpuscles was not diminished. Food consumption in the zinc fed group was ninety per cent of the food consumption of the control group which received no added zinc in the ration. The body weight of the rats which received 0.50 per cent of zinc was eighty per cent of the weight of the rats in the control group.

Female rats reared from the weaning age on a diet containing 0.50 per cent of zinc in the form of zinc carbonate showed signs of reproductive activity as indicated by the oestrus cycle. Mating was followed by the gestation period during which time pregnancy was indicated by the weight increase and the "erythrocyte sign". Litters were cast by some females following the initial pregnancy, but more generally death and resorption of the foeti occurred before the end of term. A large percentage of still born young were observed in those litters which were cast. Only a small percentage of live young were born.

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<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 497.

The oestrus activity of the female rats of the group which received 0.50 per cent of zinc was resumed following the first gestation period, and they again mated. All second pregnancies resulted in resorption of the foeti near the end of term or the birth of still born young. Positive proof of the pregnancy was obtained by observation of the "erythrocyte sign". The uteri and embryos obtained upon postmortem examination of two females, which were believed to be undergoing resorptions, indicated that the tissues were in a pathological condition. Photographs of these tissues were made.

Female rats which received 0.50 per cent of zinc in the diet ceased to become pregnant after the second pregnancy. However, when the added zinc was removed from the ration the females again became pregnant and cast normal litters. The reproductive activity of the males in the 0.50 per cent zinc group was apparently unimpaired by the zinc diet.

The volume of urine excreted by male rats which had been reared on the 0.50 per cent zinc ration was three times the volume excreted by the males of the control group. There was no evidence of abnormal excretion of reducing substances in the urine of the zinc fed rats. The blood sugar content of the rats which were fed 0.50 per cent of zinc was not abnormal.

The rats which received the 0.10 per cent zinc ration gave no indication that the ingestion of zinc at this level produced any harmful effects.

The low hemoglobin values observed in rats which were housed in screen bottom cages and fed a ration containing 0.50 per cent of zinc were definitely improved when iron chloride to the extent of 0.50 per cent of iron was incorporated into the zinc ration. The hemoglobin values improved to ninety per cent of normal or became normal in one month after the iron supplement was started.

Cadmium carbonate was incorporated into the basal ration at levels of 0.10, 0.05 and 0.025 per cent of cadmium. Groups of young rats weighing thirty to forty grams were fed the rations containing the added cadmium. The 0.10 and 0.05 per cent cadmium levels proved to be very toxic for the animals. Food consumption in the group which received 0.025 per cent of cadmium was seventy per cent of the food consumption in the control group. In the group which received 0.025 per cent of cadmium, growth as measured by the weight increase was only sixty per cent of the growth observed in the control group. The rats of the cadmium fed group became anemic as indicated by diminished hemoglobin values. The blood sugar concentration in rats of the cadmium fed group was not abnormal. The volume of urine excreted was unchanged, but the amount of reducing material excreted in the urine was increased by the 0.025 per cent cadmium diet. No sign of reproductive activity could be detected in adult female rats which were reared on the 0.025 per cent cadmium diet. A male rat from this group was shown to be fertile after 130 days on the 0.025 per cent cadmium diet.

Some changes produced by acute intoxication by zinc, cadmium and beryllium were studied. Zinc, cadmium and beryllium were administered as soluble salts to fasted rats by means of a stomach tube. The rats were anesthetized with nembutal for administration of the test solutions. Acute intoxication by zinc, cadmium and beryllium salts caused an increase in the hemoglobin concentration and an increase in the number of red blood corpuscles. Acute intoxication by the zinc, cadmium and beryllium salts

caused a fall in the blood pressure. It was found that fifteen milligrams of cadmium as the chloride, eighteen milligrams of beryllium as the sulfate and seventy milligrams of zinc as the sulfate were equally toxic when administered in one cubic centimeter of solution by stomach tube to rats.

The blood sugar concentration of rats was increased during acute intoxication produced by zinc sulfate and beryllium sulfate. It was found that subcutaneously injected calcium chloride depressed the rise in blood sugar which accompanied acute intoxication of rats by zinc sulfate. The calcium chloride was most effective in depressing the blood sugar rise which accompanied acute intoxication by zinc sulfate when the calcium chloride was injected simultaneously with the administration of the zinc salt.



# DERIVATIVES OF 1-, 4-, 6-, AND 9- SUBSTITUTED DIBENZOFURANS<sup>1</sup>

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Received July 19, 1939

By a modification of the procedure of Bywater<sup>2</sup> a 55 per cent yield of 4-aminodibenzofuran was obtained from 4-dibenzofurancarboxylic acid amide. This amine was obtained also in 45 per cent yield from a Bucherer reaction with 4-hydroxydibenzofuran. Nitration of 4-acetaminodibenzofuran in acetic anhydride at -10° had given<sup>3</sup> 3-nitro-4-acetaminodibenzofuran. When 4-acetaminodibenzofuran was nitrated in glacial acetic acid at 70° there was obtained 1-nitro-4-acetaminodibenzofuran, m. p. 216°. Hydrolysis of this nitro-acetamino compound gave 1-nitro-4-aminodibenzofuran, m. p. 219-220°, from which the amino group was removed *via* the diazo reaction and reduction with ethanol to yield 1-nitrodibenzofuran, m. p. 120-121°. Catalytic reduction of 1-nitro-4-acetaminodibenzofuran gave 1-amino-4-acetaminodibenzofuran, m. p. 202° and this upon acetylation gave 1,4-diacetaminodibenzofuran, m. p. 307-308°. The last-named compound was also prepared by amination and subsequent acetylation of 1-bromo-4-acetaminodibenzofuran (mixed m. p.). Removal of the acetyl groups by hydrolysis with hydrochloric acid gave 1,4-diaminodibenzofuran dihydrochloride, m. p. 322-323°. The free base obtained by addition of ammonium hydroxide to the solid dihydrochloride melted at 86-87°, but was too sensitive to air oxidation to be recrystallized by ordinary procedures. Nitration of 1-nitro-4-acetaminodibenzofuran gave 1,7(?) -dinitro-4-acetaminodibenzofuran, m. p. 288°, and nitration of 3-nitro-4-acetaminodibenzofuran, gave 3,8(?) -dinitro-4-acetaminodibenzofuran, m. p. 277-278°. Lithium diethylamide and 4-bromodibenzofuran reacted in ether solution to give 4-diethylaminodibenzofuran, m. p. 68-69°, the hydrochloride of which melted at 227-228°. Lithium dimethylamide furnished 4-dimethylaminodibenzofuran, m. p. 98-99°. Willis<sup>4</sup> had obtained 4-dibenzofurancarboxylic acid from carbonation of an exchange reaction between *n*-butyllithium and 4-bromodibenzofuran which was allowed to run seven and one-half hours. Comparable yields were obtained upon carbonation from ten to twenty-five minutes after mixing. From these shorter runs there was obtained, in addition to the acid and di-4-dibenzofuryl ketone, a small amount of *tri*-4-dibenzofurylcarbinol, m. p. 274-275°, the identity of which was confirmed by its synthesis from 4-carbomethoxydibenzofuran and 4-dibenzofuryllithium.

Acetic anhydride and sulfuric acid converted 2-hydroxydibenzofuran to 2-acetoxydibenzofuran, m. p. 115-116°, which upon a Fries rearrangement gave two isomeric hydroxy ketones. The predominant product was 3-acetyl-2-hydroxydibenzofuran, m. p. 168-169° (b. p. 227°/7 mm.),

<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 540.

<sup>2</sup> Bywater, Doctoral dissertation, Iowa State College, 1934.

<sup>3</sup> Gilman, Jacoby, and Swislowky, J. Am. Chem. Soc., **61**:954 (1939).

<sup>4</sup> Gilman, Willis, and Swislowky, *ibid.*, **61**:1371 (1939).

which was methylated to give 3-acetyl-2-methoxydibenzofuran, m. p. 113-114°. Oxidation of this methoxy ketone gave 2-methoxy-3-dibenzofurancarboxylic acid (mixed m. p.) The crude isomeric ketone was methylated and purified to give 1-acetyl-2-methoxydibenzofuran, m. p. 121-122°.

From 2,8-dibromdibenzofuran, prepared by an improved procedure, there was obtained upon alkaline hydrolysis 2,8-dihydroxydibenzofuran, which was converted directly to 2,8-dimethoxydibenzofuran, m. p. 88-89° (b. p. 187°/5 mm.) and to 2,8-diacetoxydibenzofuran, m. p. 150-151° (b. p. 212°/6 mm.) Cleavage of the former with hydrobromic acid and hydrolysis of the latter with hydrochloric acid gave 2,8-dihydroxydibenzofuran, m. p. 242-243°. The preparation of this compound from a biphenyl ring closure was reported recently in a patent<sup>5</sup>. Cleavage of 2,8-dimethoxydibenzofuran under milder conditions gave some hydroxy-methoxy compound which was converted to 2-acetoxy-8-methoxydibenzofuran, m. p. 110°. Hydrolysis of the last-named compound gave 2-hydroxy-8-methoxydibenzofuran, m. p. 90-91°. The preparation of this substance was reported in a recent patent<sup>6</sup>, but no constants were given. The picrate of 2,8-dimethoxydibenzofuran melted at 117-118°.

Dibromination of 2,8-dimethoxydibenzofuran gave 1,9-(?)-dibromo-2,8-dimethoxydibenzofuran, m. p. 196-197° and 3,7-(?)-dibromo-2,8-dimethoxydibenzofuran, m. p. 260-261° in a ratio of approximately two to one. Treatment of the former with *n*-butyllithium followed by carbonation gave 2,8-dimethoxy-1,9-(?)-dibenzofurandicarboxylic acid, m. p. 271-272° dec. Diazomethane converted this di-acid to 1,9-(?)-dicarbomethoxy-2,8-dimethoxydibenzofuran, m. p. 129-130°. The same di-acid (mixed m. p.) was obtained by direct metalation of 2,8-dimethoxydibenzofuran, followed by carbonation. The 3,7-(?)-dibromo-2,8-dimethoxydibenzofuran gave, upon treatment with *n*-butyllithium and subsequent carbonation, 2,8-dimethoxy-3,7-(?)-dibenzofurandicarboxylic acid, m. p. 290° dec., which was converted by methanol and dry hydrogen chloride to 3,7-(?)-dicarbomethoxy-2,8-dimethoxydibenzofuran, m. p. 183-184°. From the replacement reaction was isolated some benzoic acid, which apparently resulted from metalation of the solvent by *n*-butyllithium. This conclusion was confirmed by the isolation of benzoic acid in 5.2 per cent yield from the reaction of benzene with *n*-butyllithium.

A Friedel-Crafts reaction with 2,8-dimethoxydibenzofuran, oxalyl chloride, and aluminum chloride gave a lactone which was probably 8-methoxybenzofuro [5,6-*b*] benzofuran-2,3-dione or 9-methoxybenzofuro [5,4-*b*] benzofuran-1,2-dione, m. p. 278°. The quinoxaline derivative formed from the lactone and *o*-phenylenediamine melted at 323-325°, and methyl 2,8-dimethoxy-1 (or 3) -dibenzofuryl- $\alpha$ -oxoacetate formed from the lactone and diazomethane melted at 206-207°.

Dibromination of 2,8-dihydroxydibenzofuran gave 1,9-(?)-dibromo-2,8-dihydroxydibenzofuran, m. p. 201-202°, which upon methylation gave a compound identical with the lower melting isomer obtained from dibromination of 2,8-dimethoxydibenzofuran (mixed m. p.). Acetylation with acetic anhydride and sulfuric acid yielded 1,9-(?)-dibromo-2,8-diacetoxydibenzofuran, m. p. 173.5-174°. With the view of proving unequi-

<sup>5</sup> French patent 816,719 [C.A., 32s2145 (1938)].

<sup>6</sup> British patent 470,021 [C.A., 32:1487 (1938)].

vocally the structures of these 1,9-derivatives, the 1,9-(?)-dibromo-2,8-dimethoxy compound was converted to 1,9-(?)-dimethyl-2,8-dimethoxydibenzofuran, m. p. 106-107°, which on demethylation gave 1,9-(?)-dimethyl-2,8-dihydroxydibenzofuran, m. p. 168-169°. Three attempts to convert the dimethyl-dihydroxy compound to the corresponding diamino-compound by means of the Bucherer reaction were unsuccessful. Removal of the hydroxyl groups *via* conversion to the diamine and the diazo reaction was planned. The 1,9-dimethyldibenzofuran has been prepared by ring closure<sup>7</sup>.

Amination of 2,8-dibromodibenzofuran gave 2,8-diaminodibenzofuran, m. p. 212-213°, and acetylation of this gave 2,8-diacetaminodibenzofuran, m. p. 299-300°. These compounds had been prepared previously by another method, which had left their structures uncertain<sup>8</sup>. The previously reported melting point for the diacetamino compound was 258°<sup>8</sup>. The picrate of 2,8-diaminodibenzofuran melted at 278° dec. Bromination of 2,8-diacetaminodibenzofuran gave 3-bromo-2,8-diacetaminodibenzofuran, m. p. 259-260°, the structure of which was determined by conversion through hydrolysis and deamination to 3-bromodibenzofuran (mixed m. p.)

A Bucherer reaction with 3,4-dihydroxydibenzofuran involved only one of the hydroxyl groups and the compound obtained was probably 3-amino-4-hydroxydibenzofuran hydrochloride, m. p. 275° with darkening at 200°; the acetaminoacetoxy derivative melted at 209-210°. When 1-bromo-2-hydroxydibenzofuran was subjected to a Bucherer reaction, the bromine was displaced by hydrogen and only 2-aminodibenzofuran was obtained.

Methylation of the dibromo-2,2'-dihydroxybiphenyl of Diels and Bibergeil<sup>9</sup> gave 5,5'-(?)-dibromo-2,2'-dimethoxybiphenyl, m. p. 128-129° while acetylation with acetic anhydride and sulfuric acid yielded 5,5'-(?)-dibromo-2,2'-diacetoxybiphenyl, m. p. 105-106°.

<sup>7</sup> Sugii and Shindo, J. Pharm. Soc. Japan, 54:829 (1934) [C.A., 29:791 (1935)].

<sup>8</sup> Borsche and Schacke, Ber., 56:2506 (1923).

<sup>9</sup> Diels and Bibergeil, *ibid.*, 35:302 (1902).



# EVALUATIONS OF CONSUMPTION IN MODERN THOUGHT<sup>1</sup>

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Received July 19, 1939

Although welfare is something intangible, and no agreement on its exact components has been reached, it is usually agreed that consumption affects welfare. In other words, there is a relation between welfare and the way people use their scarce resources, such as time, energy, and money. What people have, possess, or buy, and what they do, has an effect on their welfare.

Ideas on what is wise consumption, that is, consumption conducive to welfare, can be found in history as well as in present day writing and legislation. Specific judgments of the consumption of groups of people, stated in terms of desirability or undesirability from the point of view of well-being, are termed "evaluations of consumption." If for a given historical period a sufficient number of evaluations from varied sources is analyzed, generalizations can be drawn in regard to the dominant ethical thought of that period.

To secure an accurate picture of what is wise consumption in any given period, the following requisites must be met: (1) inclusion of a variety of sources of evaluations of consumption, (2) inclusion of a representative sample of evaluations from any one source of evaluations of consumption, (3) valid interpretation of evaluations in terms of ethical thought, and (4) understanding of the nature of a culture which may be different from that in which the investigator lives.

In picturing ideas on what is wise consumption in the historical period from the fourteenth century to the present, in Europe and America, the following sources of evaluations are used: sumptuary legislation, early utopias, opinions of reformers, opinions of early economists, and religious communistic societies in nineteenth century America. For an understanding of present day ideas on what is wise consumption, particularly in the United States, evaluations of consumption are secured from studies of family scales of living and quantitative indexes of the welfare status of communities.

In the present study, while the various possible sources of evaluations of consumption are not exhausted there is a selection of sources likely to contain typical evaluations. The most important source of evaluations of consumption not discussed in this study is that of present day government regulations. Selected sources are not exhausted, but it is believed that the number of studies used in analyzing sumptuary legislation, the religious communistic societies in nineteenth century America, and family scales of living in the United States is sufficiently extensive to give a picture of evaluations of consumption. Although all quantitative indexes available on the welfare status of communities are not analyzed, a sufficient number is used to indicate the kind of evaluations such indexes contain. These evaluations are consistent with those to be found in scale of living studies.

<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 502.

Evaluations of consumption in the late medieval and early modern period show that the dominant ethical thought of that time emphasized the following ideas: (1) the right of the government to regulate personal consumption to a considerable extent, (2) the importance of maintaining social status quo, (3) the danger of innovations to well-being, and (5) the necessity of maintaining local order and military protection. The first four aspects of ethical thought are termed "ideational" and were emphasized more at that time than they are today. The fifth aspect is common to most nations in various historical periods as well as today.

Modern ethical thought in the United States, as shown in the evaluations of consumption to be found in family scale of living studies and quantitative indexes of the welfare status of communities, places the emphasis more on what people ought to do rather than on what people ought not to do. Modern ethical thought includes such ideas as (1) freedom of choice; (2) choice with knowledge, especially knowledge of how others live; and the opinion of experts, particularly scientific experts; and (3) the abundant life, materially and in terms of a variety of interests. Studies of family scales of living and quantitative indexes of the welfare status of communities, however, are inadequate in picturing modern ethical thought for they do not show to any great extent the government regulations, or lack of regulations, which imply evaluations of consumption.

Histories of the medieval and modern period contain suggestions on what influences were behind the medieval way of thinking and the rise of the modern way of thinking. Ideational ethical thought was the product of such influences as feudalism, poverty, lack of contacts between communities, and the attitudes instilled by the Catholic church. The Reformation had the immediate influence of enforcing the simple life. Influences leading to the transition to modern ethical thought include the decline of the authority of religion, rise of the authority of science, growth of democracy, increased wealth, and wider contacts among societies.

Moral arithmeticians in the eighteenth century as well as certain economists of that century and later sought to determine whether an increase in wealth causes a proportional increase in welfare. Their efforts show that little can be gained by trying to measure degrees of well-being.

The assumption of family scale of living studies is that some uses of time and some types of goods have a tendency to increase well-being. While it is possible to measure quantities of goods and uses of time, no attempt is made to measure the extent of their tendency to improve well-being.

Quantitative indexes of the welfare status of communities contain evaluations of consumption. The methodology used in such indexes involves the following procedure: The item of fact upon which data are secured pictures or portrays a certain aspect of life; that particular aspect of life is necessary to well-being; but there is no attempt to measure how essential it is to well-being. For example, expenditures for education are an item of fact picturing extent of education; there is a relation between extent of education and well-being; but no measurement is made of this relation.

Further studies of current evaluations of consumption are necessary to confirm the impression that modern ethical thought in the United States is composed of the ideas of freedom of choice, choice with knowledge, and the abundant life. Studies of evaluations of consumption in

other countries should show the nature of modern ethical thought elsewhere as compared with that in the United States. Besides specific judgments or evaluations of consumption many writers have discussed, in general terms, the question of what values are essential to welfare. This literature centers about such matters as the importance of individualism or the importance of human personality, the welfare of the nation as compared with the welfare of individuals, the position of science in relation to welfare, and the arguments for and against this or that kind of life.



## SOME ANOMALOUS FRIEDEL-CRAFTS REACTIONS<sup>1</sup>

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Received July 19, 1939

In the Friedel-Crafts alkylation of ethyl 5-bromo-2-furoate with higher alkyl halides<sup>2</sup> there is both rearrangement to a tertiary radical and a cleavage to a *tert.*-butyl radical, irrespective apparently of both the branching and length of the R group and of the nature of the X in the RX compound. This generalization is based on results obtained with *n*-amyl chloride, *n*-amyl bromide, *n*-amyl iodide, isoamyl bromide, *tert.*-amyl chloride, *n*-hexyl chloride, *n*-hexyl bromide, lauryl bromide, cetyl bromide and *n*-octadecyl bromide, each of which gave ethyl 4-*tert.*-butyl-5-bromo-2-furoate as the sole alkylated product. An alicyclic halide (cyclohexyl chloride) and an aryl-alkyl halide ( $\gamma$ -phenyl-*n*-propyl bromide) did not give any of the ethyl 4-*tert.*-butyl-5-bromo-2-furoate.

Other types of alkylating agents were tried, but, with one exception, unsuccessfully. *Tert.*-amyl alcohol alkylated after the fashion of the corresponding amyl halides. None of the ethyl 4-*tert.*-butyl-5-bromo-2-furoate was isolated in attempted alkylations with butylene, diisobutylene,  $\beta$ -*n*-amylene, cyclohexene, *p*-*tert.*-amylphenol and *tert.*-amylbenzene. The negative results with the olefins were unexpected in view of the successful alkylation<sup>3</sup> of methyl 2-furoate by butylene.

When ethyl 5-bromo-2-furoate was first treated with *n*-amyl bromide the usual cleavage and rearrangement to a *tert.*-butyl radical took place; however, the *tert.*-butyl radical replaced the bromine and entered the 5-position to give ethyl 5-*tert.*-butyl-2-furoate in yields between 31 and 40 per cent. Subsequent experiments, in which some factors were varied, showed that aluminum chloride (the catalyst) practically free of iron (a maximum of 0.02 per cent) gave ethyl 4-*tert.*-butyl-5-bromo-2-furoate but no ethyl 5-*tert.*-butyl-2-furoate. Addition of anhydrous ferric chloride to this pure grade of aluminum chloride in sufficient quantity to bring the iron content up to 0.12 per cent gave, in an *n*-amyl bromide alkylation, both ethyl 4-*tert.*-butyl-5-bromo-2-furoate and ethyl 5-*tert.*-butyl-2-furoate.

Other furan derivatives were tried in this cleavage reaction to approximate its limits and to test some theories of the mechanism. Ethyl 5-chloro-2-furoate is very similar to the bromine analog. The chlorine atom is smaller than the bromine and it was thought possible that a group larger than a butyl group might be introduced. That is, it was postulated that the cleavage was caused in part by steric hindrance and that variation of the size of the group in the 5-position should influence the size (i. e. number of carbon atoms) of the alkyl group which can be introduced. Actually ethyl 4-*tert.*-butyl-5-chloro-2-furoate was the only prod-

<sup>1</sup> Original thesis submitted June, 1938. Doctoral thesis number 473.

<sup>2</sup> Gilman and Burtner, *J. Am. Chem. Soc.*, **57**:909 (1935).

<sup>3</sup> Calloway, Doctoral Thesis, Iowa State College Library (1933); Gilman and Calloway, *J. Am. Chem. Soc.*, **55**:4197 (1933).

uct. A more significant test of the steric factor might be the alkylation of ethyl 5-fluoro-2-furoate. Unfortunately, this compound is not known.

Ethyl 4-bromo-2-furoate was tried in the reaction in order to establish whether a cleavage would take place when an  $\alpha$ -position in furan is available. It is known that there is no cleavage when methyl 2-furoate is alkylated by *n*-amyl chloride and *n*-hexyl bromide, the products being methyl 5-amyl-2-furoate and methyl 5-hexyl-2-furoate, respectively. However, when ethyl 4-bromo-2-furoate was treated with *n*-amyl chloride there was a cleavage, despite the availability of an  $\alpha$ -position, and the product isolated was ethyl 5-*tert*-butyl-2-furoate. The bromine atom must have been removed after alkylation rather than before inasmuch as ethyl 2-furoate alkylates without cleavage.

Ethyl, 4,5-dibromo-2-furoate has both the 4- and 5-positions blocked, and the remaining 3-position is very resistant to substitution. It might have been expected from results already described that a bromine atom would be replaced in a cleavage alkylation, if a halide higher than  $C_4H_9X$  were used. Actually there was no reaction with *n*-amyl chloride or *n*-amyl bromide. With *tert*-butyl bromide and isopropyl chloride there was also no reaction.

5-Bromo-2-furfural was found resistant to alkylation by *n*-amyl chloride despite the fact that 2-furfural is alkylated readily by isopropyl chloride to give 4-isopropyl-2-furfural.

An excess over one mole equivalent of aluminum chloride was found necessary to induce any alkylation at all: with cleavage or otherwise. From this it is evident that the extra quantity of catalyst required in the alkylations of the halogeno-furoates is not due to the cleavage reaction, but the first equivalent is probably tied up by a complex or coördination compound with the carboalkoxy group.

Of the several solvents examined, only carbon disulfide and *sym*-tetrachloroethane were effective. Nitrobenzene, chlorobenzene, and purified kerosene gave negative results. Negative results were also obtained when no solvent was used.

For purposes of comparison most of the reactions were allowed to continue for twenty-four hours, although much shorter periods were found satisfactory.

Preliminary to a study of the mechanism of the above cleavage alkylations it was conclusively proved that the invariable four-carbon radical does not originate from the furan nucleus. The *tert*-butyl group must then come from the alkyl halide. Cleavage to the *tert*-butyl group can either precede (or be simultaneous with) or follow alkylation. It is quite unlikely that cleavage follows alkylation since cleavage of alkylated benzenes invariably occurs at the carbon-carbon bond between the ring and the side-chain.

What may happen is that the alkyl halide, or some compound derived from it, is cleaved into fragments small enough to be introduced into the sterically-hindered  $\beta$ -position. An examination of cleavage products from the reaction between ethyl 5-bromo-2-furoate and *n*-amyl chloride showed that *n*-butane and isobutane were the chief fragments, relatively small quantities of fragments having less than four carbon atoms being involved. There was no evidence of any low molecular weight alkyl chloride, like methyl or ethyl chloride. Intermediate formation of an olefin as the active alkylating agent would help account for the forma-

tion of the highly branched tertiary radical. However, unless the olefins are in an activated state it is difficult to see how they may be the precursors in alkylation when the numerous negative results with olefins like butylene are considered.

The fragments from a reaction between ethyl 5-bromo-2-furoate, *n*-octadecyl bromide and aluminum chloride in *sym.*-tetrachloroethane consisted of butanes, pentanes, and hexanes together with some higher liquid fractions which were difficult to separate cleanly.

Paraffins are known to be cleaved by the action of aluminum chloride predominantly to *n*-butane and isobutane. The exact mechanism is not quite understood. If the alkyl halides cleave under the conditions of the furan experiments to give units of four carbon atoms, it is possible that free radicals may be the active precursors in chain reactions. These free radicals may combine, in part, with the loosened or detached nuclear hydrogen, thus accounting for the butanes isolated; and they may also combine with the free radical (or its equivalent) resulting from the bromofuroate to give the alkylated bromofuroate.

When benzene is alkylated by *n*-octadecylbromide in the Friedel-Crafts reaction the major product is the *normal*-octadecylbenzene. This was established by comparing a derivative (sulfonamide) with sulfonamides from octadecylbenzenes prepared by the Wurtz-Fittig reaction and by the reduction of stearophenone. Comparisons were made by mixed melting points.



# PREPARATION OF CHOLESTERILENE AND VARIOUS CHOLESTADIENES<sup>1</sup>

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Received July 19, 1939

The name cholesterilene refers to the compound prepared by the removal of one molecule of water from a molecule of cholesterol either by the use of various dehydrating reagents or indirectly from various cholesterol derivatives. A double bond is located in the 5,6-position (ring B) of cholesterol which possesses a negative specific optical rotation as do other cholestane derivatives with a double bond in this position. Simple dehydration of cholesterol would be expected to involve the removal of the hydroxyl group in the 3-position (ring A) with a hydrogen atom from an adjacent methylene group (4-position) to form a second double bond in the 3,4-position in conjugation with the original double bond. The cholestadiene expected would thus be 3,5-cholestadiene with a negative specific optical rotation. However the specific rotation of cholesterilene prepared by various methods has been reported with a large variation in numerical value (1). It was desired to investigate this variation in specific rotation and to compare cholesterilene with other cholestadienes.

## EXPERIMENTAL RESULTS

Cholesterilene was prepared by five different methods which were representative of the three general procedures for the preparation of this compound, namely (1) the direct dehydration of cholesterol, allocholesterol, or their epimers, (2) the removal of hydrogen halide from cholesteryl halides, and (3) the pyrolysis of cholesteryl esters. The samples obtained were purified by repeated recrystallization from different solvents and mixed solvents, by treatment with decolorizing carbon, by treatment with sodium and alcohol, and by adsorption of impurities by means of activated alumina until the melting point, specific rotation, and refractive index were unaffected by further purification.

3,5- and 4,6-Cholestadienes were prepared by the removal of two molecules of hydrogen bromide from pseudocholestene dibromide (4,5-dibromocholestane) and  $\beta$ -cholestene dibromide (5,6-dibromocholestane) respectively. Alcoholic potassium acetate was found to be ineffective for the complete removal of hydrogen bromide but quinoline was found to act satisfactorily for the conversion of the dibromocholestanes to the cholestadienes. The cholestadiene (3,5-cholestadiene) obtained from 4,5-dibromocholestane was found to be laevorotatory, which indicates that one of the double bonds is in the 5,6-position; the other double bond would be in conjugation in the 3,4-position. The cholestadiene (4,6-cholestadiene) obtained from 5,6-dibromocholestane was found to be dextrorotatory, which indicates that one of the double bonds is in the 4,5-position; the other double bond would be in conjugation in the 6,7-position.

<sup>1</sup> Original thesis submitted December, 1938. Doctoral thesis number 498.

3,5-Cholestadiene was purified by the various methods described above for cholesterol until its melting point, specific rotation, and refractive index were unaffected by further purification. It was observed during the successive stages in the purification of each sample of cholesterol and of 3,5-cholestadiene that as the melting point rose to constant value, the negative of the specific optical rotation also rose to constant value and the index of refraction became constant. The melting points and refractive indices were found to be of the same value (Table 1). The numerical agreement of the two highest laevorotations observed,

TABLE 1. *Physical properties of cholesterol (A) and 3,5-cholestadiene (B)*

| Compound | Method of preparation   | M. P.<br>in ° C. | ( $\alpha$ ) <sub>D</sub> <sup>25</sup> * | n <sub>D</sub> <sup>25</sup> ** |
|----------|---|------------------|---|---------------------------------|
| A        | Cholesterol + copper sulfate .....                                  | 79.5-80          | -104.91                                   | 1.45974                         |
| A        | Cholesterol + potassium acid sulfate ..                             | 79.5-80          | -103.91                                   | 1.45974                         |
| A        | Allo- and epiallocholesterol +<br>alcoholic hydrochloric acid ..... | 79.5-80          | -123.23                                   | 1.45974                         |
| A        | Cholesteryl chloride + quinoline .....                              | 79.5-80          | -100.24                                   | 1.45974                         |
| A        | Pyrolysis of cholesteryl methyl<br>xanthogenate .....               | 79.5-80          | -123.23                                   | 1.45974                         |
| B        | Pseudocholestene dibromide +<br>quinoline .....                     | 79.5-80          | -103.24                                   | 1.45974                         |

\* In carbon tetrachloride. The same solution was used for the refractive index determination.

\*\* n<sub>D</sub><sup>25</sup> 1.45625 for the carbon tetrachloride used.

namely those of the products obtained by the action of alcoholic hydrochloric acid upon a mixture of allo- and epiallocholesterol and by the pyrolysis of cholesteryl methyl xanthogenate, leads to the indication that the products (including 3,5-cholestadiene) obtained by the other methods still contain impurities which could not be removed by the procedure employed although the specific rotations observed are higher than previously reported (1). The treatment of cholesterol and 3,5-cholestadiene with chromium trioxide yielded oxycholestenone (cholesten-4-dione-3,6) isolated as the monophenyldrazone, which indicated their identity.

4,6-Cholestadiene was indicated not to be identical with 7-dehydrocholestene isomer (2) since 4,6-cholestadiene possessed a lower melting point and a higher positive specific optical rotation.

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# DIFFERENTIATION OF THE VITAMINS OF THE B COMPLEX AND THEIR DISTRIBUTION IN CERTAIN FOODS<sup>1</sup>

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Received July 19, 1939

Four factors of the vitamin B complex; namely, B<sub>1</sub>, flavin, nicotinic acid, and B<sub>6</sub> are known chemical entities and have been synthesized. Several additional factors in the complex have been described by various workers. The work herein reported was undertaken to obtain information on the differentiation of the factors of the B complex which are necessary for the rat. These studies have included the physical-chemical fractionation of extracts of natural foods and the study of the fractions obtained as sources of the vitamin B complex. Investigations have also been conducted on the distribution of individual factors of the complex in certain foods.

Studies were made of the preparation of lactoflavin from whey powder. One extraction with 60 per cent ethyl alcohol by volume was found more effective than three extractions with 96 per cent ethyl alcohol by volume. Adsorption of flavin directly from the 60 per cent alcoholic extract was as efficient as adsorption from the aqueous solution (after removal of the alcohol and dilution with water). Five hundred grams of fuller's earth (Cenco) per 2.25 kg. of whey powder were necessary for the most effective adsorption of flavin; the same degree of adsorption was obtained with 100 g. of English fuller's earth.

Concentrates of B<sub>6</sub> (factor 1) and factor 2 were prepared from liver and from rice polishings by a method similar to that of Lepkovsky, Jukes, and Krause (1). The B<sub>6</sub> concentrate was purified further by readsorption and elution before concentration. Both B<sub>6</sub> and factor 2 fractions were found necessary in addition to B<sub>1</sub>, flavin, and nicotinic acid for the growth and well-being of the rat.

The adsorbate and filtrate obtained by treatment of the factor 2 fraction from hog liver with norite gave no supplementary effect to one another as sources of factor 2, although about one-fourth of the total factor 2 activity was adsorbed. The unadsorbed fractions and the weakly adsorbed fractions, prepared by continuous adsorption on fuller's earth of the filtrate from flavin adsorption of hog liver extract, gave no significantly better growth when fed in combination as the source of factor 2 than when fed singly.

The feeding of 0.30 g. per rat per day of alfalfa meal as the source of factor 2 produced restricted growth (4.5 g. per rat per week) and an unhealthy appearance in rats. With the addition to the diet of 0.05 g. of fraction 8B, a fuller's earth adsorbate from rice polishings extract, almost doubled growth (8.2 g. per week) and normal healthy appearance of the animals resulted. Fraction 8B at the 0.10 g. per rat per day level, as the sole source of factor 2, was incapable of supporting growth and life; and 0.60 g. of alfalfa, as the source of factor 2, gave growth inferior to

<sup>1</sup> Original thesis submitted July, 1939. Doctoral thesis number 536.



that obtained on 0.30 g. of alfalfa plus 0.05 g. of fraction 8B. Thus it is shown that at least two components make up factor 2 and are necessary for the growth and well being of the rat, in addition to vitamin B<sub>1</sub>, flavin, nicotinic acid, and B<sub>6</sub>, as the source of the B complex. One of these components is supplied by 0.30 g. per rat per day of alfalfa; whereas, the other factor (the "8B factor") is supplied by 0.05 g. per rat per day of fraction 8B.

The 8B factor was found to be supplied by 0.30 g. of molasses per rat per day, distinguishing it from factor U of Stockstad and Manning (2). Yellow corn at a level of 0.375 g. supplied a significant amount of the 8B factor; this differentiates the 8B factor from chondroitin sulfuric acid which has been suggested as a growth factor by Robinson and co-workers (3). The 8B factor was not destroyed by heating at pH 9.0 for four hours. This stability to heat and alkali shows the 8B factor to differ from the chick antidermatitis factor, factor W, and the antiparalytic factor B<sub>4</sub>, since these three factors have been found unstable to heat and alkali by Lepkovsky and Jukes (4), Frost and Elvehjem (5), and Reader (6) respectively. At least 120 mg. of cod liver oil per day was received by each rat on the 8B factor deficient diet. This is twice the quantity of cod liver oil that Burr and Burr (7) found adequate to maintain rats for a period of a year with only slight symptoms of unsaturated fatty acid deficiency (some scales on the feet and tail but otherwise healthy). Therefore it is evident that the 8B factor is not the essential unsaturated fatty acid.

When powder and molasses were found to be poor sources of vitamin B<sub>1</sub> but somewhat better as sources of vitamin G. Molasses was found to contain about 1.6 units of vitamin B<sub>1</sub>, 3.5 units of flavin, 6.5 units of factor 2, and more than 6.5 units of vitamin B<sub>6</sub> per gram.

Rations containing white corn (0.50 g. per rat per day) or alfalfa meal (0.30 g. per rat per day) as the source of factor 2 gave better growth with the addition of nicotinic acid indicating that rats require this substance in their diet and that it is lacking in white corn and alfalfa at the levels fed.

Alfalfa meal was found to be superior to molasses as a source of factor 2 minus the 8B factor. Jukes (8) has reported that alfalfa is a poor source of the chick antidermatitis factor while molasses is a good source of it. Therefore it is apparent that factor 2 minus the 8B factor contains a component different from the chick antidermatitis factor.

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# CIRCULATION OF HEMOLYMPH IN THE WINGS OF THE COCKROACH *BLATTELLA GERMANICA* L.

## I. IN NORMAL WINGS<sup>1</sup>

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Although flow of hemolymph in wings of some insects is part of the normal circulatory scheme, many details of circulation in these appendages are yet unavailable. In spite of considerable general literature on this subject, there can still be found textbooks of entomology which state there is no circulation in the insect wing. Other publications recognize the presence of hemolymph in wing spaces, but make no mention of active circulation. For these reasons a thorough study of hemolymph movement in the wings of a typical insect may serve to bring again this activity to the attention of insect physiologists and morphologists. This is the first of a series of papers dealing with circulation in the wings of the cockroach, *Blattella germanica*. The studies should be of particular interest to entomologists since Portier (25, 26) has suggested that circulation of hemolymph through insect wings may play a part in respiratory exchange of gases, and thus may be closely linked to insect respiration.

As early as 1744 Baker (1) noted flow of hemolymph in grasshopper wings. Not until more than eighty years later did a second publication on insect wing circulation appear. In 1826 Ehrenberg and Hemprich (18) observed movement of hemolymph in wings of a mantid. From time to time since then papers have appeared with fragmentary notes or descriptions of circulation in various Orthoptera, Neuroptera, Ephemerida, Odonata, Plecoptera, Coleoptera, Tricoptera, Lepidoptera, Diptera (newly emerged), and Hymenoptera. This early literature was reviewed adequately by Yeager and Hendrickson (38) who also contributed in 1934 a description of part of the circulatory pattern in wings of *Periplaneta americana*, the large American roach. Later literature on general insect circulation has been reviewed by Hoskins and Craig (20).

Some important papers on wing circulation appearing between 1826 and 1934 are those of Carus (12, 13, 14) in 1827, 1828, and 1831; that of Verloren (35) in 1847; that of Moseley (24) in 1871; and the more recent researches of Brocher (3, 4, 5, 6, 7, 8, 9, 10, 11) published between 1909 and 1929. In 1831 Carus formulated his "rule" that hemolymph flows from the insect body through the anterior margin of the wings, and returns toward the body in the posterior margin of the wings. This early gen-

<sup>1</sup>This report was made possible through a grant from the Rockefeller Fluid Research Fund administered through Iowa State College.

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eralization has been found to hold in hexapods examined since then. The report by Yeager and Hendrickson in 1934 appears to be the latest completed with any thoroughness.

Only one note has been found on wing circulation in *Blattella germanica*. Yeager and Hendrickson merely state that circulation occurs in "elytron" (tegmen) and wing, but give no details about paths or channels in which the hemolymph moves.

#### EXPLANATION OF TERMS

The terminology of this paper is that of Needham and Comstock (17) and Comstock (16), with modifications suggested by Snodgrass (30). The more important hemolymph channels and spaces follow, for the most part, courses similar to those of wing veins (Fig. 1), and it has been convenient to use commonly accepted venation nomenclature to designate paths of circulation. Cross channels which do not follow wing veins will be referred to by names derived from the two longitudinal veins between which the channel is located. More details of wing venation nomenclature may be found in citations 1, 2, 16, 17, 21, 29, 30, 33, and 34.

The general direction of flow is described as *afferent* when the current of hemolymph is toward the distal part of the wing; and *efferent* when the direction of flow is toward the insect's body. These words are descriptive with reference to direction of flow in the wing and not to direction of flow from the propelling organ. The usage is the same in vertebrate physiology, and was adopted by Moseley (24) as early as 1871 in describing insect wing circulation.

To discuss the parts of wings concerned in circulation it is convenient to use the different regions described by Snodgrass (30): axillary, remigial, vannal, and jugal (Fig. 1). In addition, that part of the remigium with efferent flow has been called the proximal remigium, and that with afferent flow, the distal remigium. Two intermediate areas are located in the remigium: one, the distal intermediate zone, lies in the region of origin of the radial sector and the additional radial branches; the other, a proximal intermediate zone, lies at the points where efferent hemolymph from media and cubitus turns into cubitopostcubital channels.

The articular membrane and anterior sinus are important structures in circulation. The former is part of the wing which is directly connected to tergum and pleuron, and actually consists of dorsal and ventral membranous surfaces. The dorsal surface is continuous with the tergum; the ventral, with the pleuron. Anteriorly the two surfaces do not meet, and the space between them forms the anterior sinus; posteriorly, they are fused to make part of the jugum.

The basal sinus is a dilated channel in the axillary region at the base of cubitus, postcubitus, and vena dividens. It is prominent in the hind-wing, but much less pronounced in the tegmen and does not play such an active part in circulation. It should not be confused with the larger anterior sinus between the two surfaces of the articular membrane. The anterior sinus is directly connected with the hemocoel, while the basal

sinus communicates with the pulsatile organ by channels passing through the fused jugal part of the articular membrane. The basal sinus is really a dilated hemolymph channel. The anterior sinus is not, strictly speaking, a channel, but a space formed during evagination of the wing pad. Ante-

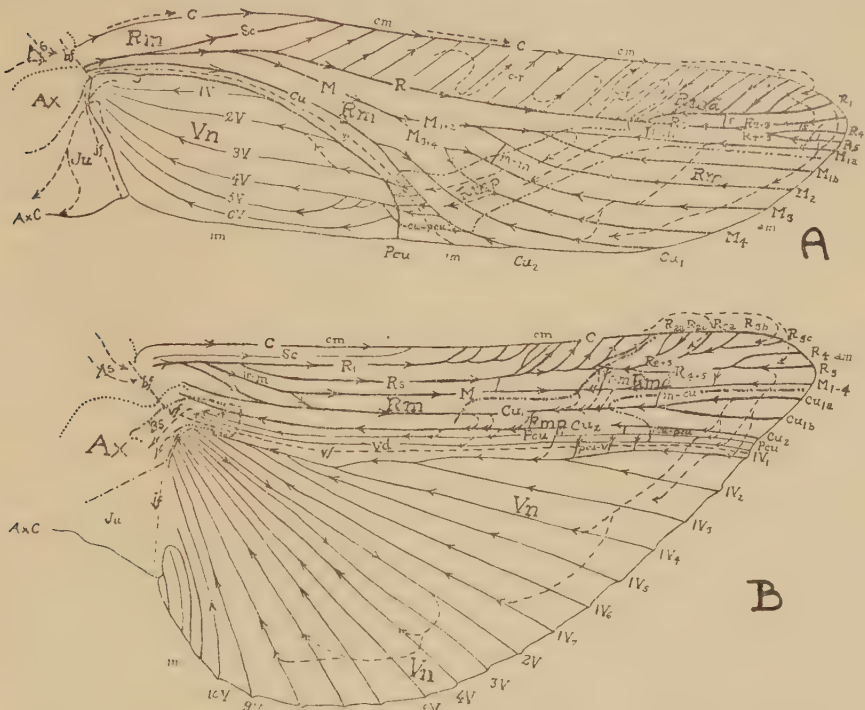


Fig. 1. Diagrams showing venation, folds, regions, sinuses, circulatory zones, and normal pattern of circulation in the tegmen (A) and hind-wing (B). Folds are indicated by broken lines; axillary regions by dot-dash lines; intermediate circulatory zones by dash-dot-dot lines; anterior sinus by line of dots; and basal sinus by dash-dot-dot-dot lines. Arrows along the veins indicate hemolymph flow in channels around or close to those veins; broken-line arrows designate general direction of flow in the many channels of the respective regions.

Abbreviations: am—apical margin; AS—anterior sinus; Ax—axillary region; AxC—axillary cord; bf—basal fold; Bs—basal sinus area; C—costa; cm—costal margin; c-r—costo-radial cross vein; Cu—cubitus; Cu-pcu—cubito-postcubital vein; im—inner margin; jf—jugal fold; Ju—jugum; M—media; m-cu—medio-cubital cross vein; m-m—median cross vein; Pcu—postcubitus; Pcu-v—postcubitus-vannal cross vein; R—radius; Rm—remigium; Rmd—distal remigial intermediate zone; Rmp—proximal remigial zone; Rs—radial sector; r—radial cross vein; r-m—radio-medial cross vein; Sc—subcosta; s—sectorial cross vein; V—vannal vein; vd—vena dividens secondary vein; vf—vannal fold; Vr—vannal region.

rior and basal sinuses are connected by the basal channel which follows the basal fold. When this channel is carrying much efferent hemolymph, especially in the hind-wing, some is directed into the vannal channels and thus accounts for the afferent streams frequently occurring in the vannal region. For additional details, see Clare (15).



Throughout this paper the circulatory paths are outlined and described with respect to the wing in an outstretched position at right angles to the body. Such procedure is necessary to obliterate folds and overlappings which occur when the wings are at rest. This extended position exposes all veins, channels, axillary membrane and its pteralia, and the connection of the wing to the body.

A normal pattern of circulation is outlined by assuming that the direction most often observed and the paths most often followed by the hemolymph are normal, based upon the original premise that the costal circulation is afferent.

Other anatomical terms are those recommended by Snodgrass (30), and used in other books on insect morphology (23, 28, 29, 33, 34).<sup>3</sup>

#### MATERIALS

Insects used for this problem were imagos of *Blattella germanica*. Adults were collected in Ames, Iowa. Some of these individuals were used for investigations until others could be reared from the original stock. They were kept in glass containers and fed canned dog food and banana. Water was always available.

#### METHOD

A method for observing circulating hemolymph was described by Yeager and Hendrickson (37) in 1933. Their procedure was to hold the insect in a depression in a balsam block by strips of paper over the pronotum and head, and over the abdomen under the wings. The strips were fastened to the wood by pins. A movable piece of tin-foil under the observed area was used to reflect light upward through the insect tissues. It frequently happens, however, that circulation is impaired in small specimens if too much pressure is applied to the papers. This difficulty was corrected by holding the roach with a thread tied loosely around the neck. A pin at each end of the thread holds the noose in position and does not interfere with circulation as much as do the paper strips. The foil strip was retained. To hold the roach close in the depression, pins also may be adjusted along the length of the neck thread, between either side of the head and the distal pin. In studying hind-wing circulation the leash was sometimes fastened between the mesothorax and prothorax.

After the roach was in position, a strong beam of light was directed on the foil under the wing and reflected through the membrane. Hemocytes could be observed in motion and directions of hemolymph circulation ascertained. The cell surfaces reflect light and make possible detection of movement. If care is taken to adjust the insect in a comfortable position it will make few efforts to escape. If the source of light is too near the microscope stage, the insect will become overheated; this may be overcome by passing the light through water.

When investigations were begun, a low power objective of a com-

<sup>3</sup> The authors are grateful to Dr. W. H. Wellhouse, Iowa State College, for his suggestions regarding the naming of the wing structures.

pound microscope was used, but a binocular dissecting microscope provided with high power objectives was later found more convenient, especially for experimental work, since much larger areas could be observed in the same field.

## RESULTS

Observations of hemolymph circulation were made while the wing was at right angles to the body. This is not the normal position, but, in most respects, gives the same circulatory pattern as the resting position. However, each possible wing position initiates very slight variations in flow and some of these changes will be pointed out later. By definition, then, hemolymph circulation in a normal wing will mean circulation in an uninjured wing held at right angles to the body in such a way that circulation is not perceptibly impaired.

### A. CIRCULATION IN THE TEGMEN

1. Axillary region: In the hemocoel or visceral sinus, the main flow of hemolymph is in a caudad direction, but some of this body fluid is continually entering the tegmen through the articular membrane. The axillary area is so constructed as to direct hemolymph from the hemocoel to the afferent channels. The two surfaces of the articular membrane are not fused in the anterior region immediately in front of the medial plates and the second and third axillary sclerites. Failure to fuse leaves an evagination or sinus (anterior sinus) between the visceral sinus and afferent channels. Pressure exerted on the hemolymph by the action of the heart propels the hemolymph through the anterior sinus, and forward into the tegmen. The fusion of the dorsal and ventral surfaces of the articular membrane posterior to the anterior sinus prevents the interference of afferent with efferent streams returning to the hemocoel. The fusion is complete with the exception of efferent jugal channels through which hemolymph passes to the axillary cord channel; and the basal channel which sometimes admits afferent hemolymph into vannal passages.

2. Remigial region: Afferent circulation is restricted mainly to the proximal remigial area of the tegmen (Fig. 1, A). Most afferent streams flow in costal, subcostal, radial, proximal medial, and cubital channels. Subcostal hemolymph is emptied into the costal channel at its distal end. The current then continues toward the outer margin as costal hemolymph. Hemolymph in the costal channel is increased by inflow of radial hemolymph from the first three or four costo-radial intermediate channels. The costal flow proceeds to the outer margin of the tegmen where, by route of cross-channels, it enters the  $R_1$  and flows to the branch of  $R_1$  and  $R_s$  and then proceeds into the medial channel together with the flow of the radius and  $R_s$ . Hemolymph current in  $R_s$  and its branches  $R_2$ ,  $R_3$ ,  $R_4$ , and  $R_5$  is usually costal in origin and enters the medial circulation just below the  $R_1$  and  $R_s$  branch by the way of the radio-medial cross-channel together with the current from  $R_1$  (Fig. 2, D).

Some radial current near the proximal end becomes diverted into

the medial and cubital channels. At the proximal end of these channels the circulation is afferent. Radial flow contributes to the costal along its way, through the first three or four costo-radial channels. The remainder of radial hemolymph passes to the junction of  $R_1$  and  $R_s$  and enters the

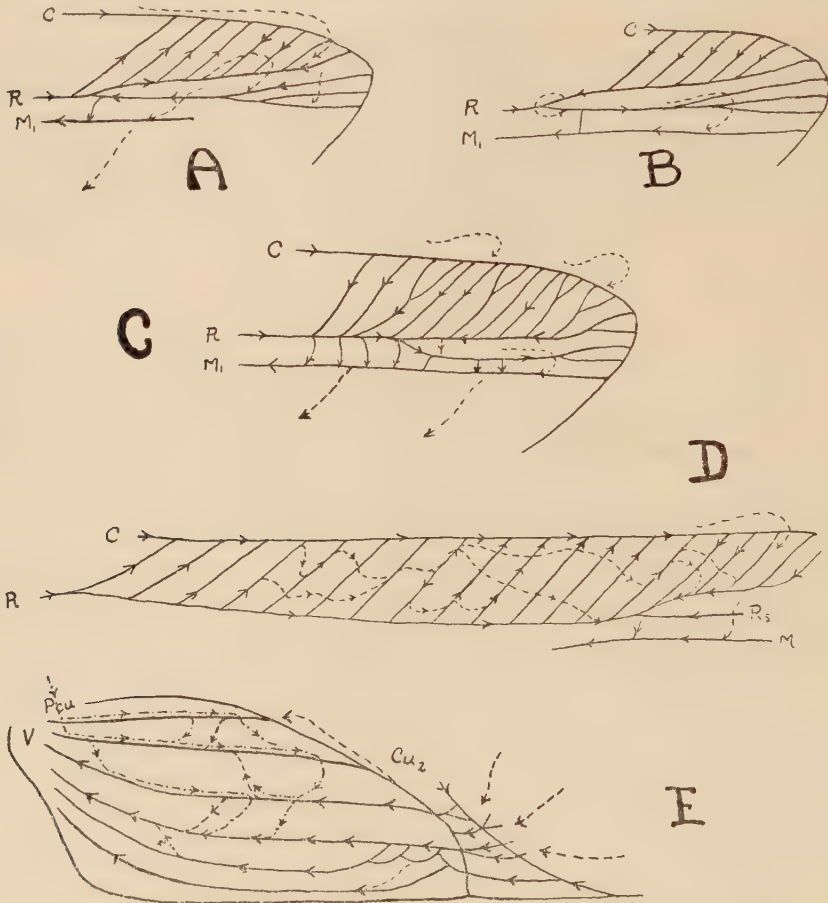


Fig. 2. Diagrams to show details of circulation in various regions of the tegmen: **A** and **B** are variations in radial currents; **C** gives details of afferent and efferent flow in the distal intermediate zone; **D** shows cubito-radial cross channels; and **E** depicts details of cubito-postcubital and vannal circulation. (In **E** dot-dash lines indicate efferent vannal currents; broken lines are cross channels in normal afferent flow; solid lines show direction of flow when all vannal currents are afferent.) (Abbreviations as in figure 1.)

medial current with the efferent flow of  $R_1$  and  $R_s$ . Except for the first three or four costo-radial cross-channels the others usually receive their hemolymph from the costal current, and the flow is not direct radial current from the radius.



The main variations in the proximal remigium lie in the costo-radial cross-channels. In the first three or four of these the flow is from radius to costa. In the remainder the flow is almost entirely costal hemolymph (Fig. 1, A). Hemolymph travels down from the costa into costo-radials beyond the first three or four, and may flow nearly to the radius. However, it does not usually enter the radial flow. Instead, the channels turn, flow through several veins distally, enter a channel corresponding to a cross-vein, and are again emptied into costal circulation (Fig. 2, D). Frequently at the origin of the  $R_s$  branch some costal hemolymph may enter the radius proper, through the costo-radials. By way of cross-channels between costa and  $R_1$ , hemolymph enters the  $R_1$  and  $R_2$  or both. This is the area in which the costal hemolymph usually becomes efferent. Actually it is one of the intermediate zones between proximal and distal zones of the remigium, and will be referred to shortly.

Medial and usually cubital currents are derived from proximal radial circulation; however, frequently the cubital channel is in direct contact with the anterior sinus, in common with the radius. Afferent flow in these channels is in the true proximal remigial region. In the medial channel the afferent circulation usually proceeds past the branching of channels of  $M_{1,2}$  and  $M_{3+4}$ . The afferent circulation of the cubitus, in both  $Cu_1$  and  $Cu_2$ , proceeds to the cubito-postcubital cross-channels (Figs. 1, A; 3, A).

Hemolymph flows from radius and  $R_s$  into distal efferent medial channels, through radio-medial cross-channels. As hemolymph proceeds along the  $M_1$  channel it is transferred by cross-channels into other branches, namely  $M_2$ ,  $M_3$ , and  $M_4$ , and courses toward the cubito-postcubital channels. Often circulation in the intermediate or cross-channels carries a large volume to  $Cu_1$  and  $Cu_2$  where the efferent circulation is usually very rapid. Proximal cubitus and media contain afferent streams. The afferent current proceeds to a point past the division of  $M_1$  and  $M_2$  from  $M_{1,2}$ , and of  $M_3$  and  $M_4$  from  $M_{3+4}$ . The cubital stream remains afferent to cubito-postcubital intermediate channels for some distance beyond the point of origin of  $Cu_1$  and  $Cu_2$ . In these two areas the two currents join and enter vannal channels by way of cubito-postcubital cross-channels (Figs. 1, A; 3, A).

Efferent and afferent currents of medial and cubital channels usually meet mid-way (Figs. 1, A; 3, A). Cross-channels then convey the two opposing currents into vannal channels. Most frequently cross-circulation commences before the efferent current reaches the point of branching of  $M_{1,2}$  and  $M_{3+4}$ . Immediately proximal to  $M_{1,2}$  and  $M_{3+4}$ , flow is afferent and meets the efferent flow distal to these points. Only extreme distal cubital flow is efferent. The opposing currents meet above the cubito-postcubital channels and with the medial current all flow directly into cubito-postcubital channels. Efferent hemolymph in  $M_1$ ,  $M_2$ ,  $M_3$ , and  $M_4$  may be transferred to the cubital afferent stream before it enters vannal circulation. As a rule, efferent hemolymph in the  $Cu_1$  and  $Cu_2$  distal to cubito-postcubital cross-channels is greater in volume than in distal medial channels.

There are two main remigial intermediate zone regions where afferent hemolymph becomes efferent. The distal zone is the area of the remigium where costal and radial hemolymph becomes efferent. The proximal zone changes medial and cubital afferent streams, with the efferent hemolymph of these channels, into one common efferent current which flows toward the inner margin of the tegmen to vannal channels. These two intermediate areas separate the tegmen into distal (efferent) and proximal (afferent) remigial circulatory regions (Fig. 1, A).

Circulation in the  $R_1$  and  $R_s$  (distal remigial intermediate zone) varies considerably. Most frequent directions are as follows: The main radial circulation turns into the radio-medial cross-channel (Figs. 1, A; 2, D), where it becomes medial efferent hemolymph. Radial afferent current stops here. Hemolymph in  $R_1$  and  $R_s$ , and branches of the latter, is hemolymph from the costal channel. Through costal-radial and radial cross-channels it is transferred to  $R_1$  and  $R_s$  where it becomes efferent in direction. These currents proceed to the radio-medial channel where they are mixed with radial flow and together enter the current of  $M_1$  (Figs. 1, A; 2, D). Sometimes some  $R_1$  current may mingle with that of the  $R_s$  branches at the sectoral cross-channel. Sectoral cross-channels connect the circulation between  $R_{2,3}$  and  $R_4$  or  $R_{4,5}$ . Some  $R_s$  hemolymph may enter  $M_1$  through several radio-medial cross-channels, but more often the main current is in a channel that corresponds closely to the commonly accepted radio-medial vein.

In several instances there have been courses different from that above (Fig. 2, A, B, C). Radial hemolymph continues afferently in  $R_1$  and  $R_s$  and its branches. Costal currents empty into  $R_1$  through costo-radials. In this example flow is efferent almost to the extreme distal part of the tegmen. The change is brought about by streams through radial and sectional cross-channels near the immediate outer margin of the remigium. Hemolymph then becomes medial through marginal radio-medial channels.

Rarely is the  $R_1$  current efferent and that of  $R_s$  is continuous with, and in the same direction, as the main radial current (Fig. 2, B, C). In this case flow in  $R_1$ , which is costal hemolymph, turns at the origin of branches  $R_1$  and  $R_s$  and mingles with the radial and  $R_s$  afferent stream. This current is transferred to the afferent medial channel by radio-medial intermediate channels near the apical margin of the tegmen. The true radio-medial channel corresponding to the radio-medial cross-vein plays little part in this scheme.

3. Vannal region: In the tegmen this region is separated from the remigium by the postcubitus (Figs. 1, A; 2, E). Normally there is no circulation in this channel except distally where hemolymph may flow forward and enter one of the first vannal channels. Vannal circulation is an efferent flow, most of which passes through two or three channels more directly connected with the cubito-postcubital cross-channels: namely, 4V, 5V, and sometimes 6V. All vannal channels converge near the base of the region at the third axillary plate to which the vannal veins

are connected. Currents of hemolymph are transferred along this plate into the jugal region or membrane.

All vannal channels seldom take part in efferent circulation at one time. If hemolymph proceeds up the postcubitus, there will be some efferent circulation in 1V, 2V, or 3V. Otherwise circulation along their full course is not frequent. Along the proximal ends much of the streams of 4V, 5V, and 6V may enter these channels by cross-channels. Very often the entire efferent flow enters 4V, and from this is dispersed to the other vannal channels. Sometimes there may be some afferent circulation in the vannal channels of the normal wing. In such cases hemolymph is admitted by the afferent basal channel. The basal channel carries afferent streams into the basal sinus which is in turn connected with two or three of the first vannal channels. This afferent hemolymph turns in the distal vannal area to become part of the efferent stream. Efferent circulation is, however, not the rule, but may be brought about very easily experimentally, and will be described in a later paper.

Cubito-postcubital channels are those through which all remigial hemolymph of the tegmen must pass to gain entrance to the pulsatile organ. Their number varies, but as a rule there are not more than five, of which two or three are generally active. These cross-channels convey hemolymph past the postcubitus into one or two vannal channels.

4. Jugal region: This portion of the wing is part of the articular membrane. Its two surfaces are fused in a plane in the same manner as the fusion of the two wing surfaces. In the jugum are one or more channels which pass from the entrance of the vannal current, below the third axillary, to the caudal margin of the jugal membrane along which the axillary cord passes (Fig. 1, A). These channels allow passage of hemolymph from vannals to the axillary cord.

5. Tergal (notal) region: When hemolymph enters the axillary cord channel it flows directly to the mesothoracic pulsatile organ, then into the heart or dorsal sinus and forward toward the head. The axillary cord is a membranous structure continues with the caudal margin of the jugum and tergum. This "cord" channel along the tergum is functionally separate from and not continuous with the dorsal sinus. It provides a channel for transport of hemolymph from the jugal area to the mesothoracic pulsatile organ. As far as can be determined, this tergal cord is structurally continuous with the intersegmental membrane, but fused along a line to prevent its continuity with the hemocoel. The direction of hemolymph flow in the hemocoel just beneath the intersegmental membrane is in a mesad direction.

Many of the circulatory paths indicated above vary with affecting conditions. At one time a channel may be entirely stagnant or even empty, while at another moment hemolymph will circulate actively.

#### B. CIRCULATION IN THE HIND-WING

1. General description: Hemolymph flow in the hind-wing (Fig. 1, B) is in most respects similar to that in the tegmen, and, therefore, does



not need detailed description. Hemolymph enters the costa and a single basal channel which branches into the subcosta, radius, media, and proximal  $Cu_1$ . The bases of  $Cu_1$  and  $Cu_2$  and postcubitus channels usually have a "blind" origin and, as in the tegmen, the postcubital channels are not directly connected with the hemocoel. The  $Cu_1$  derives its afferent hemolymph from the media.

The stream in the costa, subcosta, radius, media, and first cubital branch (proximal) is afferent (Fig. 1, B). The subcosta empties into the costa nearly mid-way along the costal wing margin. The radius distributes afferent hemolymph to the costa and proximal media. Costal and radial flow turns efferently into the distal efferent sections of the media,  $Cu_1$ ,  $Cu_2$ , postcubitus, and vena divicens from where it passes in one of two directions. Part of the flow frequently crosses the vannal fold and vena divicens into the first branch of 1V (Fig. 1, B); the remainder continues efferently along the  $Cu_2$ , postcubitus, and vena divicens emptying into a large channel (basal sinus) at their bases (Fig. 3, B). Here a portion of it may travel afferently in some vannals (usually 2V and 3V) and return in other vannals (4-10V) with more active currents proceeding in an efferent direction. The remainder passes directly through the jugal channels. Usually that which crosses into the first branch of 1V from the cubitus is sufficient to supply the efferent flow for the 1V<sub>1</sub> to 1V<sub>7</sub> branches of the 1V. Some may flow to 4-10V before turning efferently. Frequently the entire efferent flow in 4-10V may be derived from the basal sinus through afferent flow in 2-3V channels.

#### C. DIFFERENCES IN CIRCULATION IN THE TWO WINGS

Although the pattern of hemolymph flow in the two wings is much alike, several variations should be mentioned. In the tegmen afferent currents are found sometimes in one, two, perhaps more, of the first vannal channels. For some roaches this streaming seems to be a normal course; however, it has been found to vary even in such individuals. Experimentally this afferent current is very easily established as will be shown in a later paper. This hemolymph flows directly from the hemocoel through the basal fold channel into the vannals. On entering vannal channels it flows afferently and joins efferent currents coming from cubito-postcubital channels. When these first few channels do not have this afferent current they are often not functional, but at any moment currents may be initiated in them and pass efferently toward the body. Apparently a local increase in volume of hemolymph necessitates this. It is strange, however, that in very active circulation afferent currents are less often observed.

In the hind-wing (Figs. 1, B; 3, B; 3, C) afferent currents in the vannal area are more commonly observed, and may be considered a normal course. There are two sources for this hemolymph. One is that hemolymph from the basal fold channel which is identical with the occasional path described for the tegmen. Currents usually enter 2V, 3V, and sometimes one or two others. The current passes to the margin

of the wing and turns efferently. The other, and more important, source of this afferent hemolymph is from efferently returning streams from the remigium. Part of this volume passes efferently down  $Cu_2$ , postcubitus and vena dividers to the basal sinus in the axillary region but does not all

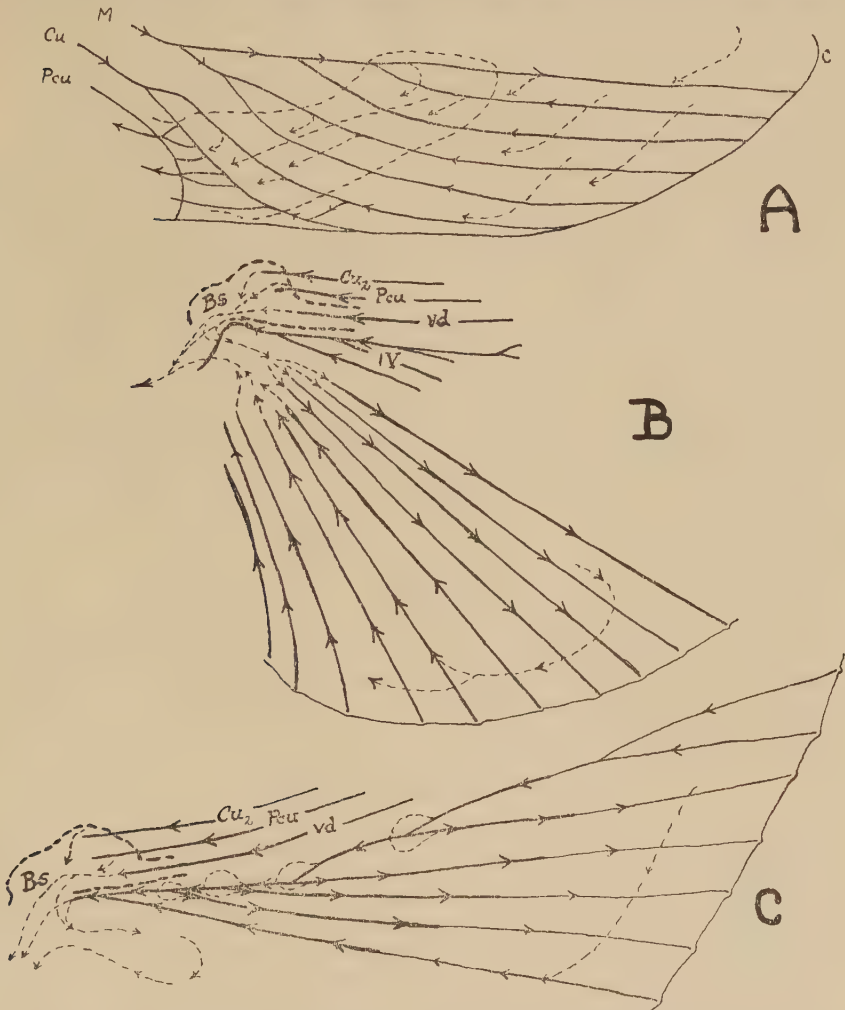


Fig. 3. A shows afferent circulation in proximal intermediate zone of tegmen. B and C show a part of the hind wing. B indicates entry of hemolymph into the basal sinus and the peculiar efferent course into the upper vannal channel. C diagrams a variation in the first vannal channel. (Abbreviations as in figure 1.)

pass into the jugal channels. This efferent current along the  $Cu_2$ , postcubitus and vena dividers is a very common path for the return of efferent remigial hemolymph, and it is exceptional to see a roach that does not have it.

Another difference is found in the pattern of remigial flow. In the hind-wing all efferent remigial hemolymph does not pass over  $Cu_2$ , postcubitus, and vena dividers to gain entrance to vannal currents (Fig. 1). If hemolymph succeeds in passing over cubito-postcubital and postcubito-vannal channels into the vannals, it passes down to the jugal area mostly by way of  $1V$  channels. Some of this hemolymph continues to the pulsatile organ, as does all remigial hemolymph in the tegmen, while some may pass with cubital and postcubital efferent flow into afferent vannals. The latter volume reaches the pulsatile organ through efferent vannals.

Sometimes there may be afferent currents in the hind-wing in branches of  $1V$  (Fig. 3, C). This is due to diversion of cubito-postcubital and postcubito-vannal currents passing into vannal channels. The stream passes some distance down  $1V$  and turns efferently in  $1V_2$ ,  $1V_3$ , and sometimes all branches except  $1V_1$  which maintains an efferent flow. Afferent hemolymph then crosses to vannal channels carrying efferent currents.

After noting these variations it is important to point out that frequently currents in all hind-wing vannals may be in the same direction.  $Cu_2$ , postcubital, and vena dividers currents pass directly to the jugum, and hemolymph which passes into vannal channels over cubito-postcubital and postcubito-vannal paths maintains an efferent flow in the entire vannal area. Only one constant difference thus occurs in the two wings. This arrangement lies within  $Cu_2$ , postcubitus, and vena dividers channels. An afferent current has never been observed in the proximal  $Cu_2$  channel of the hind wing, while in the tegmen it is always afferent to cubito-postcubital channels. In the basal postcubital channel, currents are always present in the hind-wing, but in the tegmen this path seems to be non-functional, proximally.  $Cu_1$  in both wings is alike in maintaining part efferent and part afferent streams. This arrangement allows division of either wing into two circulatory zones separated at the place where most of the afferent currents become efferent.

#### D. REVERSAL OF CIRCULATION

Reversal of circulation in the tegmen as a whole is very rare in the German roach. Often under condition of abnormal external pressure, circulation in remigial afferent channels becomes efferent. As a general rule under such conditions vannal circulation is not reversed. This may be explained by assuming a low pressure area about the base of remigial channels so that hemolymph may flow back into the hemocoel.

In the hind-wing reversal of flow in remigial channels is fairly common under the least abnormal strain on the wing while at rest, or during flight movements. Reversal seems to be caused by the same pressure lowering as suggested for the tegmen. Vannal circulation may or may not reverse, depending on duration of reversal in the remigium. Frequently when a wing was drawn out of position there occurred a sudden rush of hemolymph into the remigial afferent channels. This change has been called rush movement. It seems to be accompanied by contraction of abdominal and thoracic muscles whereby intra-hemocoelic pressure be-



comes abnormally high and hemolymph rushes into the wing. Excess hemolymph is forced into the wing and accelerates vannal flow. After release of pressure the general flow of the afferent channels becomes efferent and much of the hemolymph returns from the remigium to the hemocoel. During this reversal the vannal flow continues in the normal direction. This movement is periodic and has not been observed to follow rhythmic contractions of the heart. It is most frequently observed in newly emerged adults.

An efferent current in the usual afferent remigial channels, as explained above, is the result of varying pressures at the base of the wing. Vannal circulation is rarely reversed. If vannal circulation is reversed simultaneously with that of the afferent remigial, observations indicate this reversal is also due to changes in the normal hemolymph pressure areas. Abnormal heart pressure might propel hemolymph afferently through vannal channels that are normally efferent, but there is considerable evidence that the pulsatile organ is not continuous with the heart, and, if this be true, a reversal in heart beat would not necessarily cause reversal in wing circulation, especially in vannal channel flows. Observational evidence gained in this study implies that reversal in the vannal circulation is due to an abnormal pressure gradient established by a decreased pressure on afferent remigial currents. This decrease may result from retarded heart action, or from relaxation of thoracic and abdominal muscles, or from a combination of these factors.

#### DISCUSSION

Study of the wings of German cockroaches clearly shows that these membranous appendages possess a flow of hemolymph which is directed along rather definite courses which have been called hemolymph channels. These channels differ from the true blood vessels of vertebrates in that the former are sinusoidal extensions of the hemocoelic cavity. They may be rather narrow and confining, or quite broad and extensive.

In these channel spaces, hemolymph enters the anterior or costal margin of the wings and flows distad in an afferent direction to the apical margin of the wings where it turns and travels toward the body in an efferent direction. The main streams of hemolymph are in channels which follow the course of the wing veins. Some channels surround trachea, whereas others do not accompany tracheal branches. The latter is the condition in the cross-channels and costal channel.

The wings are always provided with many more channels than are functional at any one time. When higher hemolymph pressure prevails or when certain main channel flows are increased or accelerated, hemolymph is directed through spaces which were previously empty. This arrangement allows the courses in specific areas to become altered, although the general circulation continues to follow a definite circuit. The presence of channels which are sometimes non-functional is by no means an indication of indefiniteness and random flow in insect wing circulation; in the vertebrate capillary system there are many inactive vessels which

become functional under varying physiological conditions, and the circulatory paths in vertebrates are considered "stable."

General directions of afferent and efferent currents seem essentially alike in all insects studied, and confirm Carus' (14) rule that hemolymph enters and flows along the costal wing margins, and streams toward the body along the inner wing margin. When patterns of wing circulation from *Blattella germanica* and *Periplaneta americana* are compared, many similarities are seen. The differences between these related species are minor and are brought about by different venation in the two sets of wings.

Researches so far conducted indicate that many Pterygota have circulating hemolymph in their wings: some throughout life; some in early imago stages only. Some insects in which wing circulation has not been demonstrated may in reality possess currents in these appendages. This failure to demonstrate circulation may have arisen from the method of study. Many insects have low hemocyte counts (31, 32) and circulation of the clear plasma cannot be noted until a cell or particle moves along. In order to see circulation in such insects, injection methods must be used or long periods of very careful observation are necessary.

In some insects there appears to be wing circulation only in early imago stages. Carus records circulation in a newly emerged fly, *Eristalis apiformis*. This observation has not been verified, and it is not known whether other Diptera are similar in this respect. In such orders as Orthoptera, Coleoptera, Ephemerida, and Neuroptera wing circulation has been more fully studied and it is probable that most insects of these orders maintain a wing circulation. A table prepared by Yeager and Hendrickson (38) lists the insect species in which circulation has been observed.

Circulation in insect wings may have functions more than superficial in nature. Portier (25, 26) has shown that painting wings of Lepidoptera with vaseline; removing wings; severing the pulsatile organ which aids circulation from the wing; or cutting wing tracheae cause the insect to fatigue rapidly. This behavior is perhaps due to disequilibrium of gaseous exchange in the respiratory system. It may be either impaired adjustments to intake of oxygen or to diffusion of carbon dioxide. The latter impairment is more likely, and it is quite probable that wing hemolymph takes part as a liquid medium in the diffusion of gases. Physically, wing surfaces provide a good mechanism for diffusion, and since they approach being membranes with properties necessary for gaseous diffusion, they provide areas much more efficient than the general body wall. If carbon dioxide is transported in the body in a carbonate-carbonic acid system, it is possible that hemolymph could readily release carbon dioxide while circulating through the wing.

Surfaces such as furnished by the wings might also provide for diffusion of oxygen into hemolymph and allow its combination in a physical sense as stated in Henry's law: the solubility of a gas in a liquid is directly

proportional to the pressure of the gas, provided there is no chemical union of the gas and the liquid.

During the present investigations microscopic bubbles of gas were observed in the wing channels when hemolymph flow was stopped. Frequently under such conditions the tracheae were collapsed. A plausible physiological explanation might be that the gas was carbon dioxide which was released from tracheae but could not diffuse out of the wing. The wing surfaces, themselves, probably were not greatly altered, but the stagnant hemolymph did not offer conditions for rapid diffusion. The loss of wing surfaces as organs aiding in elimination of respiratory wastes would cause carbon dioxide or carbonates to accumulate in the hemolymph. Such accumulation might slow down general body activities due to change of hydrogen ion concentration which regulates the thoracic respiratory centers (36). Although this mechanism is implied by behavior of the insect under experimental conditions, much more work will need to be done before definite conclusions can be drawn.

Other experimenters also have shown that the wing fluid can absorb certain substances which are then carried into the hemocoel. Portier (25) found that application of sub-lethal doses of nicotine to wings of *Lepidoptera* was followed by "convulsions" characteristic of nicotine poisoning. Applications of stronger solutions caused death. Richardson, Glover, and Ellisor (27) exposed wings with an active circulation to nicotine vapors. After two hours the bodies of such cockroaches contained sufficient nicotine to be measured quantitatively. These results indicate a definite permeability of wing surfaces. They also show that hemolymph going through the wing spaces takes up these substances and carries them to the central nervous system as well as to other regions. Surely a gas with diffusibility properties such as possessed by carbon dioxide would find it easy to leave the hemolymph through the wing surfaces. Consequently the wing and its hemolymph may contribute to respiratory exchange in some hexapod forms. This would be of particular aid in flying insects whose wing movements provide constantly changing volumes of air around the wing surfaces.

Another function of wing hemolymph must be to carry food materials necessary for maintenance of wing tissues. It is very likely that the nutritional composition of the wing hemolymph is like that of the hemocoelic fluid. Epidermal cells may be relatively few in wings of the mature adult, but complete absence does not occur. The wing is not a "dead" structure; it is a living organ and, as such, makes the usual demands of living tissues. Clumps of epithelial cells are located in the channel spaces; in some places they may form the channel boundaries. Stoppage of circulation causes the wings to curl, dry, and become brittle. If the flow of hemolymph is impaired in the wing of a newly emerged, unpigmented adult, the structure becomes dry, brittle, malformed, and lacks normal pigmentation.

The real function of the pulsatile organ has not yet been worked out. This organ was not studied in these investigations from an anatomical



standpoint, and conclusions in this paper concerning it are derived from its action in the living roach. Meyer (22) made a close anatomical study of the ephemerid naiad and found that the hemolymph flow through the pulsatile organ was continuous with the wing circulation. He found that hemolymph entered the pulsatile organ from the wing pad and was emptied directly into the dorsal sinus and not into the heart. In the ephemerid naiad the folds of the heart are such that when it dilates and fills with hemolymph, the membrane of the heart wall is forced against the pulsatile organ, and the contained hemolymph is expelled through valvular flaps into the dorsal sinus. When the heart contracts a pressure gradient ("suction") is set up between the pulsatile organ and wing channels and hemolymph flows into the organ. The dorsal sinus hemolymph is prevented from re-entering the organ because it rushes back against and closes the pulsatile organ valves. Pulsatile organs of the honeybee and other insects have also been discussed by Brocher (3) and Freudenstein (19). In the German roach the course of circulating fluid to the pulsatile organs is essentially the same as that described by Meyer (22) for the May-fly naiad. Vannal hemolymph passes into the jugal region, collects at the posterior border of the jugal region, collects at the posterior border of the jugal membrane, and flows down the axillary cord which is continuous with a membrane lying along the posterior border of the notum. From all observations so far, there is no evidence that this channel is continuous with the dorsal sinus. Hemolymph flows from the jugum and axillary cord through this area directly into the pulsatile organ. It was not possible to determine the fate of the pulsatile hemolymph, but it probably is expelled into the dorsal sinus as described for the immature May-fly. There is evidence that the organ in some insects is continuous with the heart cavity. In writing of heart beat reversal in the American roach, Yeager and Hendrickson (38) state that circulation in the wing is also reversed. This correlation indicates probable continuity of pulsatile organ and heart in this species. In the German roach the relationship is somewhat different. Complete reversal of wing circulation was very seldom observed and seemed due mainly to reduced pressure in the afferent channels in the remigium. This reduction may result from temporary cessation of heart action; sudden slowing of heart rate; or relaxation of abdominal and thoracic muscles. There is little evidence that the reversal is correlated with heart reversal. This relation indicates the continuity of the dorsal sinus and pulsatile organ as in the May-fly naiad. Reversal of wing circulation as a whole was noted so rarely in the German roach that the writers are inclined to regard it an abnormal condition regardless of whether the organ is continuous with the heart or dorsal sinus. Reversal might also occur under abnormal strains in the animal when subjected to observational and experimental conditions.

#### SUMMARY

The cockroach, *Blattella germanica* L., has been used to ascertain the actual courses of wing circulation in an insect of its type, noting particu-

larly the definiteness with which streams of hemolymph are maintained, and the way in which the complete circuits are directed by the structures present. Suggestions relating to the functions of wing circulation are also presented.

Hemolymph currents enter the wing channels along the anterior margin. These afferent streams are transferred by several cross-channels into efferent currents which carry hemolymph toward the pulsatile organ. The actual flow of hemolymph is directed by the arrangement of structures in the base of the wing. Here the dorsal and ventral surfaces of the articular membrane are not in contact, and thus allow the hemolymph to enter the afferent channels. Beyond this open area the two surfaces are fused except at points where the afferent channels originate. These, in turn, connect with cross-channels which are, under normal conditions, the only means of returning hemolymph to the hemocoel by way of the efferent streams. Efferent streams enter the hemocoel through jugal fold channels which communicate with the pulsatile organ. The general scheme of circulation is similar in both fore- and hind-wings and differs only in details resulting from somewhat different patterns of venation in the two wings. Details of circulation are shown in figure 1.

The articular membrane and its contained structures provide a mechanism by which currents of hemolymph are maintained under a pressure gradient. The anterior wing channel streams are under a pressure which gradually decreases as the flow passes into the cross-channels. In the vannal channels further decrease in pressure is brought about by the "sucking" action of the pulsatile organ as indicated by Meyer in his report on the May-fly (22). Hemolymph is thus brought into the pulsatile organ which returns it to the general body cavity. Fusion of the articular membrane about the vannal channels prevents interference by hemocoelic pressure. Heart contractions and hemocoelic pressure apparently are responsible for the action of the pulsatile organ.

This study of wing circulation has brought out many questions regarding actual function of circulating body fluid in the wing channels. It has been suggested that circulation in these appendages is directly concerned with respiration. Transport of nutritive material to the wing tissues is another important function. Wing circulation is also demanded in keeping wings in their normal, healthy condition. In addition, the shifting patterns and volumes of the wing channels in response to various activities of the body indicate their dynamic role in the physiological activity of the organism as a whole.

#### CONCLUSIONS

1. An active circulation of hemolymph occurs in the tegmina and hind-wings of the German cockroach, *Blattella germanica* L.
2. In general, the main afferent currents are restricted to costal and radial channels, and to proximal medial and cubital channels. The important efferent channels are in distal medial and cubital channels, and vannal channels. These afferent and efferent routes, especially pro-

nounced in the tegmina, are connected by intermediate cross-channels. This general pattern is not so well defined in the hind-wings where direction of flow varies more widely.

3. Certain constant differences in circulatory paths exist between tegmen and hind-wing:

a. In the tegmen the proximal postcubitus is not active; in the hind-wing it usually carries an efferent stream.

b. In the tegmen the proximal remigial intermediate zone connects afferent and efferent currents of the media and cubitus, and returns the hemolymph to the pulsatile organ by way of the vannals; in the hind-wing not all the hemolymph is transferred through the proximal intermediate zone into the vannals at the distal extremity. Some passes efferently along the  $Cu_2$ , postcubitus, and vena dividers, and then empties into the basal sinus. From there it may either enter vannal channels at their base and pass afferently, or the basal sinus hemolymph may go directly to the pulsatile organ. Hemolymph which passes afferently in the vannals will return to the jugum by more posteriorly situated vannals. Most of the hind-wing remigial flow to the vannals through the proximal remigial zone passes toward the jugum in the seven branches of the first vannal channel.

4. Secondary currents—that is, temporary flow in certain channels—may occur in either tegmina or hind-wings, though not as often in the former. In the tegmen they occur only in the vannal channels and carry hemolymph directly from the hemocoel, through the basal fold channel, into the vannals (Fig. 2, E). The same basal channel flow may be found in the hind-wing (Fig. 3, B). In addition, other secondary currents in the hind-wing are due to cubital and postcubital flow from the remigium (Fig. 3, C). These are distinct from currents originating in the hemocoel and going directly to the vannals through the basal fold.

5. The anterior separation and posterior fusion of dorsal and ventral surfaces of the articular membrane are important in maintaining hemolymph flow through the wings. This arrangement in the membrane, along with the action of the pulsatile organs, apparently make possible a pressure difference between out-flowing hemolymph and in-flowing hemolymph. The anterior separation (anterior sinus) allows direct continuity between hemolymph in the hemocoel and afferent wing channels, whereas hemolymph in efferent channels has connection with the main volume of body fluid only through the valvular pulsatile organ. It is possible, and has been suggested by other writers, that the pulsatile organ aspirates hemolymph from the efferent channels. If this is true, it would make for further pressure differences between afferent and efferent wing hemolymphs at their contact with the thoracic hemolymph.

6. Circulation of hemolymph through the wings of the German roach is necessary to maintain the wings in a flexible condition and to bring about normal pigmentation. Since the wings contain various tissues, hemolymph undoubtedly carries nourishment to, and wastes away from, these tissues. Wings with impaired circulation often contain bubbles of



gas. It is suggested that these are an indication of interference with exchange of respiratory gases. Thus the passage of gases through wing surfaces may be closely related to respiration in the roach as well as in certain Lepidoptera.

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## FUNGI ASSOCIATED WITH TREE CANKERS IN IOWA II. DIAPORTHE, APIOPORTHE, CRYPTODIAPORTHE, PSEUDO- VALSA AND THEIR RELATED CONIDIAL FORMS<sup>1</sup>

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Received October 13, 1939

In a previous paper (7) a preliminary report was made of a survey of the fungi associated with tree cankers in Iowa. Since that time more intensive study has been made upon some of these collections, and the genus *Diaporthe*, with the related genera, *Apioporthes*, *Cryptodiaporthe*, and *Pseudovalsa*, have been reinvestigated in the light of the recent monograph of this genus by Wehmeyer (20). All species in these three genera which have been found in Iowa are reported; together with the species of the form-genera *Phomopsis* and *Coryneum*, which are associated with *Diaporthe* and *Pseudovalsa* as conidial stages.

In the genus *Diaporthe*, as in many other genera of Ascomycetes, there has been very little accord among mycologists as to the species limits. Since *Diaporthe* is the ascigerous stage of *Phomopsis*, which causes serious disease conditions in a wide range of host plants, a tendency arose to place too great significance on host relations and to erect new species whenever an unreported host was found. Wehmeyer (20), having studied the species in culture, has founded his treatment of the species entirely upon morphological grounds and has simplified the mycological situation and cleared the way for further contributions in this group.

The genus *Diaporthe* as emended by Wehmeyer (20) contains those species of the *Diaporthaceae* in which the perithecia are formed within the substratum of the host and break through to the surface by means of a more or less elongated perithecial neck. These perithecia may be scattered singly over the surface, irregularly clustered, or definitely oriented into groups, within an area which shows, at least at some points, a marginal blackening of the tissues. Asci are clavate to clavate-cylindric, with a refractive ring in the thickened apical wall, and sessile. They are soon freed from their attachment by dissolution of the basal portion, and then lie free within the perithecium. Paraphyses are broad, band-like; present at first but disappearing with maturity. Spores are fusoid-ellipsoid to cylindric, straight, inequilateral or curved, two-celled, hyaline, sometimes appendaged and bi- or uniseriated in the ascus.

The conidial stage is referable to the form-genus *Phomopsis*, characterized by its pycnidium which has a rather thick, blackened pseudoparenchymatous wall and by the presence of two types of conidia, the ellipsoid conidia (alpha spores) and the long scolecoform stylospores

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<sup>1</sup> Journal Paper No. J-687 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 450.

(beta spores). These pycnidia are usually formed in the ectostromata before the perithecia are present.

Twelve species of *Diaporthe* have been collected in Iowa; *Diaporthe arctii* (Lasch.) Nit., *D. eres* Nit., *D. spiculosa* (Alb. and Schw.) Nit., *D. viburni* Dearn. and Bisby, *D. prunicola* (Pk.) Wehm., *D. decedens* (Fr.) Fckl., *D. padi* Otth., *D. acerina* (Pk.) Sacc., *D. pruni* Ell. and Ev., *D. leiphaemia* var. *Raveneliana* (Thüm. and Rehm) Wehm., *D. tessella* (Pers.) Rehm., and *D. tiliacea* (Ell.) Höhn.

The genus *Cryptodiaporthe* as emended by Wehmeyer (20) includes those species of *Diaportheaceae* which possess a rather weak stromatic development, and have no blackened zones within the substratum. Conidial stages may be variously formed.

The genus *Apioporthe* as emended by Wehmeyer (20) includes those forms which have perithecia like *Diaporthe*, but in which the entostromatic development is scanty with no marginal zones within the substratum. The ectostroma is variously formed. The spores are hyaline and unequally two-celled.

*Pseudovalsa*, on the other hand, usually shows a well-developed marginal zone about the clustered perithecia. The spores are hyaline or colored and more than two-celled. They may be appendaged.

The descriptions of the species of *Diaporthe*, *Cryptodiaporthe* and *Apioporthe* were taken from Wehmeyer (20), those of *Pseudovalsa* from Wehmeyer (18) and those of *Phomopsis* from Diedicke (1) except *P. stewartii* Pk., *P. mali* Roberts, *P. vexans* (Sacc. and Syd.) Harter, and *P. juniperovora* Hahn which were taken from the descriptions by the original authors.

A key to the Iowa species, followed by their descriptions:

- A. Conidia present.
  - B. Conidia in pycnidia; of two sorts ..... 5. *Phomopsis*
  - B. Conidia in acervuli; of a single sort ..... 6. *Coryneum*
- A. Ascospores present.
  - B. Ascospores more than two-celled ..... 4. *Pseudovalsa*
  - B. Ascospores two-celled
    - C. Stroma without blackened marginal zones ..... 2. *Cryptodiaporthe*
    - C. Stroma with blackened marginal zones
      - D. Ascospores equally divided ..... 1. *Diaporthe*
      - D. Ascospores unequally divided ..... 3. *Apioporthe*
- 1. *Diaporthe*
  - A. Perithecia scattered ..... *Effusae*
  - B. On herbaceous stems ..... 1. *D. arctii*
  - B. On woody stems
    - C. Spores 1.5-3 $\mu$  in diameter ..... 5. *D. prunicola*
    - C. Spores 2.5 $\mu$  or more in diameter.
      - D. Spores 9-15 $\mu$  in length.
        - E. Perithecia scattered ..... 2. *D. eres*
        - E. Perithecia clustered.
      - F. Spores fusoid ..... 3. *D. spiculosa*



- D. Spores 12-18 $\mu$  in length.
  - E. Perithecia scattered, 240-400 $\mu$  in diameter ..... 4. *D. viburni*
  - E. Perithecia tending to be clustered, 480-720 $\mu$  in diameter ..... 6. *D. decedens*
- A. Perithecia gathered into groups ..... *Pustulatae*
- B. Ectostromata strongly developed as a cone or pulvinate grayish disk
  - C. Ventral zone definitely present in bark or wood ..... 9. *D. pruni*
  - C. Ventral zones not present in wood ..... 10. *D. leiphaemia*
- B. Ectostromata poorly developed
  - C. Spores not more than 5.5 $\mu$  in diameter.
    - D. Entostromata limited in area. Perithecia definitely clustered ..... 8. *D. acerina*
    - D. Entostromata not limited in area. Spores straight ..... 7. *D. padi*
  - C. Spores reaching in diameter greater than 5.5 $\mu$ 
    - D. Spores appendaged ..... 11. *D. tessella*
    - D. Spores not appendaged ..... 12. *D. tiliacea*

## 2. *Cryptodiaporthe*

- A. A single species ..... 1. *C. aculeans*

## 3. *Apioportha*

- A. Stromata small, as a minute disk ..... 1. *A. apiospora*
- A. Stromata larger, elongate, 1-5 x 12 mm. .... 2. *A. anomala*

## 4. *Pseudovalsa*

- A. Spores fusoid ..... 1. *Ps. longipes*
- A. Spores rounded at ends, appendaged ..... 2. *Ps. ulmi*

## 5. *Phomopsis*

- A. On herbaceous hosts.
  - B. On *Plantago* ..... 1. *Ph. subordinaria*
  - B. On *Cosmos* ..... 2. *Ph. stewartii*
  - B. On *Solanum* ..... 3. *Ph. vexans*
  - B. On conifers
    - C. On *Juniperus* ..... 4. *Ph. juniperovora*
    - C. On *Pinus* ..... 5. *Ph. conorum*
  - B. On deciduous hosts.
    - C. On *Acer* ..... 6. *Ph. acerina*
    - C. On *Pyrus* ..... 7. *Ph. mali*
    - C. On *Salix* ..... 8. *Ph. salicina*

6. *Coryneum*

- A. On deciduous hosts
  - B. On *Quercus* ..... 1. *C. kunzei*
  - B. On *Castanea* ..... 2. *C. pustulatum*
- A. On conifers
  - B. On *Juniperus* ..... 3. *C. juniperinum*

1. *DIAPORTHE* (Nit.) Wehm.

Perithecia formed within the substratum, erumpent to the exterior through an ectostroma or directly through the overlying tissues by means of a more or less elongated perithecial neck, scattered singly, irregularly clustered or in definitely oriented groups, formed within an area of entostromatic development which shows a marginal blackening of the tissues, at least at some points. Asci clavate to clavate-cylindric, with a refractive ring in the thickened apical wall, sessile, soon freed from their attachment by dissolution of the basal portion and coming to be free within the perithecium. Paraphyses broad, bandlike, present at first, but disappearing with maturity, spores fusoid-ellipsoid to cylindric, straight, unequilateral or curved, two-celled, hyaline, sometimes appendaged, and biseriate to uniseriate in the ascus.

1. *Diaporthe arctii* (Lasch) Nit.

Plate I. Figs. 1-4

Entostromata normally widely effuse and indefinitely outlined. Appearing on the surface as slightly, irregularly or heavily blackened, widely effuse or confluent areas. Dorsal blackened zones usually developed on the bark surface but often masked by the overlying epidermis. Ostioles cylindric, erumpent singly or in small loose groups. Ventral zone present at least at the margin of the fruiting areas. Perithecia spheric or somewhat flattened,  $280-480 \times 160-320\mu$ , usually buried in the wood. Asci clavate, with a refractive ring in the apex,  $47-60 \times 7-10\mu$ . Spores biseriate, fusoid-ellipsoid, straight or more or less inequilateral or curved, two-celled, hyaline, constricted at the septum when mature,  $12-15 \times 2.5-4\mu$ .

On *Asclepias syriaca* L. *Decorah*: Holway July, 1884; (*Diaporthe asclepiadis* Ell. & Ev.) (4)

On stems of some composite plant. *Decorah*: Holway July, 1892.

On *Asclepias syriaca* L. *Decorah*: Holway July, 1884; (*Diaporthe eburensis* Sacc.) (4)

On dead stem of *Chenopodium* sp. *Ames*: Gilman March, 1925. (*Diaporthe euspina* (C. & E.) Sacc.)

2. *Diaporthe eres* Nit.

Plate I. Figs. 5-8

Entostromata widely effuse, usually over wide areas. Appearing upon the surface as small pustulate ruptures or angular perforations of the

periderm, often exposing the blackened surface of the bark or ectostromata. Ostioles short cylindric, erumpent singly or in small loose clusters. Surface of the bark usually blackened, ventral zones always present at the margins of the fruiting areas and usually more or less complete beneath. (In the Iowa specimens the ventral zone is absent.) Perithecia spherical or flattened  $240-800 \times 160-500\mu$ , scattered singly, buried in either the bark or the wood. Asci clavate, with a refractive ring in the apex,  $40-60 \times 5-8\mu$ . Spores biseriate, hyaline, long, narrow-fusoid, or often inequilateral, two-celled, fusoid, constricted at the septum,  $9.5-15 \times 2.5-4\mu$  at maturity.

On *Cornus paniculata* l'Her. Decorah: Holway June, 1892. (*Diaporthe cornicola* Ell. & Holw.) (5)

On *Acer* sp. (soft maple): Iowa City, G. W. Martin Oct. 1924.

### 3. *Diaporthe spiculosa* (Alb. & Schw.) Nit.

Plate I. Figs. 9-12

On surface as compact circular, pustulate disks of cylindric ostioles. Stromata widely effuse. Dorsal zone often faint or definitely absent. No ventral zone present. Perithecia  $200-500\mu$  in diameter, definitely clustered in small groups and collectively erumpent. Asci clavate, with refractive ring in the apex,  $40-47 \times 6-9\mu$ . Spores biseriate, fusoid-ellipsoid, two-celled, hyaline,  $12-15 \times 2.5-4\mu$ .

On *Juglans cinerea* L. Decorah: Holway July, 1882. (*Valsa bicincta* C & P. Holway June, 1892. (Ell. & Ev. N. A. F. 2817.) (*Diaporthe bicincta* (C & P.) Sacc.)

### 4. *Diaporthe viburni* Dearn. & Bisby.

Plate I. Fig. 13-16

Appearing on the surface as numerous crowded, papillate pustules, 0.1-0.2 mm. in diameter, through which short ostioles are barely erumpent, singly or in small groups. Dorsal blackening entirely absent or present merely as a blackening above the perithecia, or as short patches of a dorsal zone dipping into the bark. No ventral zone present. Perithecia  $240-320\mu$  in diameter, thickly but irregularly scattered, erumpent singly or in small groups. Asci clavate, with a refractive ring in the apex,  $55-67 \times 8-12\mu$ . Spores biseriate, fusoid-ellipsoid to oblong-ellipsoid, two-celled, hyaline, slightly constricted at the septum at maturity,  $14-18 \times 4-6\mu$ .

On *Viburnum lentago* L. Decorah: Holway June, 1882. (*Diaporthe Beckhausii* Nit.)

### 5. *Diaporthe prunicola* (Pk.) Wehm.

Plate I. Figs. 17-20

Appearing on the surface as circular or laterally elongate, pustulate ruptures of the periderm, through which are erumpent the compact disks composed of fascicles or stout-cylindric, punctate ostioles. The surface of the bark is characteristically blackened and covered with numerous small,



black, carbonaceous papillae. The entostromata are evenly effuse, with the dorsal blackening on the bark surface, but the clustered perithecia cause pustulate swelling of the entostromata. There is a ventral zone in the wood. Perithecia 400-720 x 320-600 $\mu$ , in definite clusters within pustulate swollen areas of the entostroma. Asci clavate, 40-55 x 6-8 $\mu$ . Spores biseriate to triseriate, oblong-cylindric, with rounded ends, hyaline and one-celled at first, becoming two-celled at full maturity, straight or slightly curved, very slightly if at all constricted, four guttulate, 11-13 x 1.5-2 $\mu$ .

On *Prunus* Sp. *Decorah*: Holway May, 1892. (*Diaporthe Pruni* Ell. & Ev.)

On *Prunus americana* Marsh. *Decorah*: Holway May, 1888. (*Diaporthe cylindrospora* Pk.)

#### 6. *Diaporthe decedens* (Fr.) Fckl.

Plate II. Figs. 25-28

When young, appearing on the surface as numerous small, circular, pustulate, ectostromatic disks 0.2-0.4 mm. in diameter, through which the papillate ostioles become separately erumpent, forming scattered groups. No blackened zone occurs in either bark or wood. Perithecia spherical or flattened, 480-720 x 320-480 $\mu$ , in loose clusters but always separately erumpent. Asci clavate, with a refractive ring at the apex, 65-85 x 10-15 $\mu$ . Spores fusoid ellipsoid, and variable in size, 13-22 x 3.5-6 $\mu$ . Faint hyaline appendages are sometimes present on the spores.

On *Corylus* sp. *Decorah*: Holway, June, 1892. (Ell. & Ev. N.A.F. 2818)

This species was also reported from Iowa by Ellis and Everhart (4) as *Diaporthe tessera* (Fr.) Fckl.

#### 7. *Diaporthe padi* Otth.

Plate II. Figs. 35-38

On the surface as circular to fusoid, pustulate disks 0.2-1 mm. in diameter, consisting of a compact cluster of short, stout, cylindric, punctate ostioles. Entostromata normally pustulate-effuse, sometimes evenly effuse when the perithecial clusters are crowded, rarely isolate. Dorsal zone dipping to the bark between perithecial groups. Ventral zones absent, greenish or yellowish discolorations sometimes seen in the wood. Perithecia usually definitely clustered, sometimes irregularly crowded, 320-640 x 240-400 $\mu$ , often causing pustulate swelling of the entostromata, collectively erumpent. Asci clavate, with a refractive ring in the apex, 60-75 x 7-10 $\mu$ . Spores biseriate, fusoid-ellipsoid, two-celled, hyaline, constricted at the septum, 13-19 x 2.5-4 $\mu$ .

On *Prunus hortulana* Bailey Ames: Miller September, 1924. (*Diaporthe pruni* Ell. & Ev.)

#### 8. *Diaporthe acerina* (Pk.) Sacc.

Plate II. Figs. 39-42

Appearing on the surface as numerous pustulate ruptures of the periderm through which the short, stout-cylindric ostioles are barely erumpent

in small loose clusters. Entostromata pustulate-effuse, more or less differentiated, widely extended or often more or less limited in area. Dorsal zone definite, dipping into the back between the perithecial groups. Ventral zone definite and complete within the wood. Perithecia  $300-500 \times 225-300\mu$ , irregularly scattered or in loose groups within the pustulate areas but usually collectively erumpent. Asci clavate with a refractive ring in the apex,  $60-73 \times 7-10\mu$ , spores biseriate, fusoid-ellipsoid, two-celled, hyaline, not constricted except at full maturity,  $12-15 \times 3-5\mu$ .

On *Acer saccharinum* L. *Grand River*: Stark September, 1933.

Wehmeyer (20) states that this species is confined to *Acer spicatum* Lam. Our material is most closely like the descriptions of this species. Very possibly the host was erroneously determined by the collector.

### 9. *Diaporthe pruni* Ell. & Ev.

Plate II. Figs. 29-34

Appearing on the surface as pustulate, conic, mostly laterally elongate stromata with a central circular to elliptic, whitish to blackened disk,  $1-1.5 \times 0.3-0.7$  mm. in diameter, through which the cluster of cylindric ostioles is erumpent. Entostromata pustulate-effuse, dorsal zone dipping to the wood between the perithecial clusters and inclosing the lighter-colored, differentiated, entostromatic areas, within which the perithecia are clustered beneath a well developed grayish to greenish ectostroma. Ventral zone definite within the wood. Perithecia  $500-700 \times 300-500\mu$ , usually partly or entirely sunken in the wood. Asci clavate,  $65-92 \times 6-10\mu$ . Spores biseriate, fusoid-ellipsoid, two-celled, hyaline, constricted at the septum,  $15-20 \times 4-5.5\mu$ , on with a short thick, hyaline, evanescent appendage,  $4-8\mu$  in length, at each end of the spore.

On *Prunus* sp. (Black cherry). *Decorah*: Holway May, 1892.

The species of *Diaporthe* on *Prunus* have been much confused. Of the three collections cited by Gilman and Archer (6) under *Diaporthe pruni* Ell. & Ev. only one remains under this name. The other two are referred to *D. padi* and *D. prunicola*, respectively.

### 10. *Diaporthe leiphaemia* var. *raveneliana*

(De Thuem. and Rehm.) Wehm.

Plate II. Figs. 21-24

Appearing upon the surface as well developed pulvinate ectostromata which have ruptured the periderm in an angular fashion, exposing the erumpent, circular to angular orange-yellow to brownish disks, which become cracked and roughened in age. Ostioles scarcely erumpent. No blackened dorsal zone was apparent. Perithecia radially elongated  $240-480 \times 320-640\mu$ , definitely clustered beneath the ectostromatic disks and surrounded by a rich development of ectostromatic mycelium. Asci clavate with a refractive ring in the apex,  $55-65 \times 6-9\mu$ . Spores biseriate, fusoid, ellipsoid, hyaline, two-celled, slightly constricted at the septum,  $12-15 \times 4-6\mu$ .

On *Quercus* sp. *Decorah*: Holway, May 1833 (*Valsa leiphaemia* Fr.)

This species was reported from Iowa as *Diaporthe taleola* (Fr.) Sacc. by Ellis and Everhart. (4)

### 11. *Diaporthe tessella* (Pers.) Rehm (4)

Plate III. Figs. 43-45

Visible on the surface as clusters of separately erumpent, black, papillate ostioles, which are usually groups about a minute perforation of the periderm which exposes a blackened ectostromatic disk 0.2-0.4 mm. in diameter. The perithecia are irregularly spherical, 400-725 $\mu$  in diameter and occur singly or in groups of 2-8 within pustulate stromatic areas 1-4 mm. in diameter. A sharp black zone extends from the margins of the area into the bark tissue. When the entostromata are isolated, this dark zone continues along the lower bark surface between stromata. There is no ventral zone within the wood. The perithecia are separately erumpent through the periderm. Asci clavate, with a refractive ring at the apex, 110-115 x 18-21 $\mu$ . The spores are fusoid-cylindric, usually curved or bent at the septum, two-celled, hyaline, constricted at the septum, 35-55 x 7-9 $\mu$ , and often possess a faint, short, hyaline appendage at each end.

On *Salix* sp. *Decorah*: Holway May, 1892.

### 12. *Diaporthe tiliacea* (Ell.) Höhn.

Plate III. Figs. 46-48

Appearing on the surface as numerous minute conic, pustulate ruptures of the periderm exposing a disklike cluster of short or somewhat elongated, cylindric black ostioles. Disks 0.1-0.6 mm. in diameter. Perithecia spherical or radially elongated, 320-600 x 320-560 $\mu$ , grouped in clusters of 3-15 within slightly differentiated entostromatic areas, which are usually bounded by a more or less definite dorsal and lateral blackened zone, which is irregularly pustulate about the confluent stromata. There is usually a broad brownish discolored ventral zone within the wood. Asci clavate, with a refractive ring in the apex, 90-120 x 13-20 $\mu$ . Spores biseriate, fusoid-ellipsoid, straight or slightly curved, constricted at the septum, 24-39 x 6-9.5 $\mu$ .

On *Tilia americana* L. *Decorah*: Holway May, 1892 (Ell. & Ev. N.A.F. 2826).

This species was described by Ellis (3) from Iowa material collected by J. C. Arthur at Ames, October, 1882, as *Diatrype tiliacea*; later Ellis and Everhart (4) transferred it to the genus *Melanconis*.

## 2. CRYPTODIAPORTHE (Petrak) Wehm.

Perithecia immersed in the bark, more or less irregularly scattered or in definite clusters, but usually with convergent ostiolar necks which are erumpent through the periderm or through variously formed ectostromata. Ectostromata scantily developed or as definite conic to pulvinate erumpent disks, or as a loose web of hyphae causing broad angular ruptures of the



periderm. Entostromata very scanty or as a rich development of hyphae about the perithecia, often forming definitely oriented stromata. No blackened marginal zones within the substratum. Asci clavate, often with a refractive ring in the apex and with a tapering base which is evanescent. Spores hyaline, two-celled, ellipsoid to fusoid, straight or curved and often appendaged. Conidial stages various.

1. *Cryptodiaporthe aculeans* (Schw.) Wehm.

Plate III. Figs. 53-56

Appearing upon the surface as pustules containing dense fascicles of elongate-cylindric ostioles, erumpent through a disk which may be obliterated by the ostioles. Perithecia are spherical,  $260-480 \times 250-400\mu$ , with long slender necks and thickly clustered beneath the ectostromatic disks. No blackened zones occur in the substratum. Asci clavate,  $47-65 \times 5-8\mu$ . Spores biseriate, long fusoid-ellipsoid, two-celled, hyaline, constricted at the septum,  $12-18 \times 2.5-3\mu$  and usually with a short, hyaline appendage at each end.

On *Rhus glabra* L. Decorah: Holway July, 1884. (*Valsa culeata* Sz.)

3. APIOPORTHE (Höhn.) Wehm.

Perithecia immersed in the substratum, usually clustered. Entostromatic development scanty or variously developed as a mycelial weft or definite stromatic tissue about the perithecia. Ectostromata also various. Tissues above the perithecia sometimes blackened, but no definite marginal zones within the substratum as Diaporthe. Asci clavate, with a refractive ring in the apex, stalks evanescent. Spores hyaline, unequally two-celled, fusoid to pyriform, commonly tapered toward one end, which contains the smaller cell. Conidial stage consisting of variously shaped cavities formed within a stromatic pycnidial tissue by the simultaneous breaking up of the hyphae, in these locular areas, into conidia without the formation of a definite hymenium.

1. *Apioporthes apiospora* (Ell. & Holw.) Wehm.

Plate III. Figs. 49-52

Barely visible on the surface as minute pustules with a small central, blackened disk composed of a cluster of a few barely erumpent ostioles. There are no blackened zones within the substratum. Perithecia  $240-320\mu$  in diameter, found in small groups in the surface layers of the bark. Perithecial walls thick ( $24-35\mu$ ), membranous, black. Walls of several perithecia often fused together to form a stroma-like structure. Ostioles collectively erumpent. Asci  $75-80 \times 8-10\mu$ . Spores obliquely uniseriate to sub-biseriate, ellipsoid-ovoid, narrower toward one end, unequally two-celled hyaline constricted at the septum,  $11-14 \times 2.5-5.5\mu$ .

On *Ulmus* sp. Decorah: Holway May, 1892. (*Diaporthe apiospora* Ell. & Holw.)

2. *Apioportha anomala* (Pk.) Höhn. (4, p. 531)

Plate III. Figs. 57-58

Pustules prominent, longitudinally elongate blackish disks, rupturing the periderm, 2-5 mm. in length and about 2 mm. in diameter, and showing the blackened circular, slightly sulcate ostioles. Perithecia elongate crowded in the blackened stroma and reaching to the wood. Asci clavate,  $33-42 \times 10-12\mu$ . Spores biseriate, ellipsoid with a somewhat pointed end which is composed of a minute cap-like second cell,  $10-12 \times 4\mu$ .

On *Corylus americana* Walt. Decorah: Holway September, 1882.

Compare Ell. & Ev. N.A.F. 1185.

Ellis and Everhart (4) report this fungus from Iowa as *Cryptospora anomala* (Pk.) E. & E.

## 4. PSEUDOVALSA Ces. &amp; Denot.

Ectostromata strongly developed, dark colored, Entostroma within the area of bark tissue, at first eliminated by a blackened marginal zone. Ascospores more than two celled, and two to three seriate within broad clavate asci. The conidial stage is referable to the form genus *Coryneum*.

1. *Pseudovalsa longipes* (Tul.) Sacc.

Plate III. Figs. 61-64

Appears on the surface as small conical pustules, caused by the formation of the small ectostromatic disk beneath the periderm. The periderm is ruptured irregularly and the blackened disk with the small papillate ostioles is exposed but scarcely erumpent. Perithecia small, spherical,  $320-450\mu$  in diameter, surrounded by a blackened zone of tissue. Asci are stout to elongate-clavate  $100-150 \times 13-25\mu$ . Spores 2-3 seriate in the ascus, long cylindrical, straight or somewhat curved, acute at the ends at first, finally blunt; hyaline at first, becoming brown at maturity; 4-8 celled, constricted at the septa at maturity,  $45-75 \times 5.5-11\mu$ .

Conidial stage consisting of large acervuli with conidia, cylindric-clavate to irregular-fusoid, straight or variously curved or bent, yellow-brown, 3-8 septate,  $47-104 \times 10-14\mu$  and belonging to the form genus *Coryneum* and referred by Tulasne (16) to *Coryneum kunzei*.

On *Quercus imbricaria* Michx. Albia: Burnett October, 1933.

On *Quercus rubra* L. Ledges State Park: Lee September, 1933.

2. *Pseudovalsa ulmi* Wehm. (22)

Plate III. Figs. 65-67

Appears on the surface as small conical pustules 1-1.5 mm. in diameter caused by the formation of the ectostromatic disk beneath the periderm. The center of the disk is raised at the center by the compact cluster of black papilliform ostioles. Perithecia small, 6-10 in a pustule,  $300-350\mu$  in

diameter entirely submerged in the bark and not penetrating to the wood. Asci clavate to obovate  $75-150 \times 25-60\mu$ , obscurely paraphysate, 8-spored. Spores inordinate, oblong, slightly curved and subinequilateral, four-celled  $25-45 \times 18-22\mu$ , hyaline, at first; on maturity the center cells become olive brown with lighter end cells, each with a cylindrical hyaline, straight or curved appendage,  $12-20 \times 5-6\mu$ .

On *Ulmus fulva* Michx. Atlantic: Butler September, 1933.

## 5. PHOMOPSIS Sacc.

Pycnidia with a broad base, lens-, barrel-, bolster-form to spherical, from sclerotial tissue, hyaline below, darkened and thicker above, with variously shaped mouths. Locules often divided into partial chambers by lateral or basal protrusions of the tissue. Spores elongate, ovoid, or fusoid, typically with two oil-drops. Sporophores thread-like or pear-shaped, usually longer than the spores, hyalin. In addition to these spores there occur in some species thread-like spores which are curved or hamate.

### 1. *Phomopsis subordinaria* (Desm.) Trav.

Plate V. Figs. 78-81

Stems of the host plants bleached over the affected area, very often sharply bent upwards at the tip. Pycnidia gregarious, often arranged almost serially, elongated in the direction of the longitudinal axis of the stem; in section lens-shaped, formed of sclerotial tissue, dark red-brown, thicker at the apex, with an ostiole; about  $500\mu$  long,  $240\mu$  wide,  $150\mu$  high. Alpha spores spindle-shaped, with two oil drops,  $7-9\mu$  long,  $2-3\mu$  thick. Sporophores pear-shaped,  $15\mu$  long,  $1.5\mu$  thick, hyaline.

On *Plantago aristata* Michx. Conesville: Archer 1927.

Wehmeyer has shown this fungus to be the conidial stage of *Diaporthe arctii*.

### 2. *Phomopsis stewartii* Pk.

Plate V. Figs. 82-85

Pycnidia gregarious, commonly occupying grayish or brown spots, thin subcutaneous, at length erumpent, depressed, minute,  $1/3-1/2$  mm. broad, black; spores of two kinds, alpha spores oblong or subfusiform, hyaline, commonly binucleate,  $8-12 \times 2-3\mu$ ; beta filiform curved, flexuous or uncinat, hyaline  $16-25 \times 1-1.5\mu$ ; sporophores slender, equal to or shorter than the spores.

On *Cosmos* sp. (cult.) Homestead: Archer and Haskell 1927.

On *Cosmos bipinnatus* Cav. Ames: Elmer 1924.

The ascigerous stage of this fungus has been found, as reported at the Pittsburgh meeting of the American Mycological Society by A. L. Harrison, to be closely related to *Diaporthe phaseolorum*. He has since described it as *Diaporthe stewartii* (10).



3. *Phomopsis vexans* (Sacc. and Syd.) Harter

Plate V. Figs. 86-89

On the foliage and stems, pycnidia loosely gregarious in more or less definite spots, on fruit compact, at first buried, later erumpent, black without, beaked, flattened or irregular in shape, on leaves and stems 60-200 $\mu$  broad, on fruit 120-350 $\mu$  broad; Alpha spores subcylindrical, somewhat acute, continuous hyaline, two guttulate, rarely three, 5-8 x 2-28 $\mu$ ; beta spores filiform, curved, rarely straight, 13-28 $\mu$  long. Sporophores simple short, straight or slightly curved.

On *Solanum melongena* L. (cult. eggplant) *Muscatine*; Porter, 1920.

Harter (12) notes in his paper on the *Diaporthe* on lima bean that the *Phomopsis* on eggplant is very like the conidial stage of *Diaporthe batatatis* Harter and Field. Wehmeyer (20) reduces this latter species to a variety of *Diaporthe phaseolorum*.

4. *Phomopsis juniperovora* G. Hahn

Plate V. Figs. 90-93

Pycnidia lens-shaped, conical, truncate or at times subglobose with a broad base, 124-310 $\mu$  in diameter. (Upon the stem of the pycnidia may have rudimentary partitions arising from the floor of the cavity.) Alpha spores oblong or ellipsoid, with subacute extremities, usually biguttulate, hyaline, continuous, 6.5-12 x 1.8-3.5 $\mu$ ; beta spores, long filamentous, curved or hooked hyaline 20-24 x 1-1.5 $\mu$ . Sporophores filiform, linear, 2-15 x 1-2 $\mu$  in compact layers.

On *Juniperus virginiana* L. Ames: Archer 1927; Shenandoah: Wilson 1925.

5. *Phomopsis conorum* (Sacc.) Died.

Plate IV. Figs. 94-96

Pycnidia ectostromatic, not associated with a perithecial entostroma, simple or compound, imbedded, partially erumpent; black carbonaceous, cone-shaped, lenticular, subglobose or truncate, with or without a definite ostiole; inner pycnidium of the simple fruit body; unilocular, cavity formed in one plane with a thickened layer of pseudoparenchymatous tissue above and with protrusions from the side wall and the base of the fruit body, hymenium lining the cavity more or less convoluted giving rise to sporophores; compound pycnidium; multilocular, chambers also tending to form and fuse in one plane, 0.1-2 x 0.1-0.8 mm. Spores of three types, alpha, beta and intermediate; alpha type hyaline, unicellular, generally spindle-shaped with acute or subacute, gently rounded extremities, rarely elliptical, extreme range 6.2-14.6 x 2.5-4.7 $\mu$  commonly 7.5-12.4 x 2.5-3.7 $\mu$  with two guttules; beta type hyaline unicellular, filamentous, pronouncedly curved, hamate horseshoe-shaped rarely straight, commonly 20.2-24.1 x 1 $\mu$  with several minute guttules.

- On *Pinus strobus* L. Henry Co.: Hendershott September 1933. Garfield: Sylvester 1933. Page Co.: Ellis September, 1933.  
On *Pinus sylvestris* L. Winnebago Co.: Beatty Dec., 1933.

6. *Phomopsis acerina* Wehm.

Plate V. Figs. 74-77

Pycnidia thickly gregarious, often touching and almost confluent, covered by the epidermis, erumpent only at the tip, irregularly lens-shaped, sometimes with band-shaped ostiolar papillae, of black-brown, interiorly bright, sclerotial tissue, up to  $500\mu$  in diameter. Alpha spores spindle-shaped with two oil drops,  $8-10\mu$  long,  $3\mu$  thick, hyaline. Sporophores thread or pear-shaped, a little longer than the spores. Beta spores were not observed.

On Acer sp. (cult. maple seedlings) Shenandoah: Muncie and Bliss 1927.

This species was identified by Gilman and Archer (6) as *Phomopsis lebiseyi* (Sacc.) Died. which is the conidial stage of *Cryptodiaporthe lebiseyi*. However, Wehmeyer has confined that species to a European distribution and refers the American species to *Diaporthe acerina* (Pk.) Sacc. Our pycnidial stage answers the description of the *Phomopsis* described by Wehmeyer (20) for this species. Hence we are referring to it under the name *Phomopsis acerina* Wehm.

7. *Phomopsis mali* Roberts

Plate V. Figs. 97-100

Pycnidia subglobose, scattered, gregarious or in a stroma, black, carbonaceous plurilocular, ostiolate. Alpha spores subfusoid, containing two oil drops, continuous hyaline,  $7-10 \times 3-4\mu$ . Sporophores awl-shaped,  $20 \times 2.5\mu$ . Beta spores thread-like, hooked or S-shaped, attenuate,  $20-36 \times 1.5\mu$ .

On *Pyrus malus* L. Ames: McNew March, 1934.

8. *Phomopsis salicina* (Westend.) Died.

Plate V. Figs. 101-102

Pycnidia gregarious, covered by the epidermis, often confluent or bound in a stroma-like tissue, broadly lens-shaped or flat barrel-shaped, of sclerotial tissue somewhat thicker above, divided into semi-locules by partial floor and lateral partitions. Alpha spores long-spindle-shaped  $6-7 \times 2-2.5\mu$ , hyaline. Sporophores fasciculate, as long as the spores, thread-like. Beta spores were not observed.

On Salix sp. Atlantic: Butler September, 1933.

6. CORYNEUM Nees.

Acervulus shield-shaped, or cushion shaped, erumpent through the bark, black, compact. Spores elongate, spindle or club-shaped, with two

or more septa, brown, not found in cirrhi. Conidiophores rod-shaped, of various lengths.

### 1. *Coryneum kunzei* Corda

Plate IV. Fig. 64

Acervuli shield-shaped, flattened, erumpent, with thick black-brown, sclerotial parenchymatic basal stroma which becomes almost hyaline above, up to 1 mm. broad. Spores sub-clavate, spindle-shaped, somewhat curved, with seven oil drops and 6-9 septa, 60-70 $\mu$  long, 12-14 $\mu$  broad, dark olive brown, with the somewhat truncate end-cells usually hyaline. Sporophores thread-like, septate up to 120 $\mu$  long, 3-4 $\mu$  broad.

On *Quercus* sp. *Decorah*: Holway 1882.

On *Quercus alba* L. *Albia*: Alstatt 1933; *Creston*: Noecker October, 1933.

On *Quercus imbricaria* Michx. *Mt. Pleasant*: Hendershott, May, 1933.

On *Quercus rubra* L. *Ames*: McNew September, 1933; *Des Moines*: Parsons September, 1933.

On *Quercus velutina* Lam. *Creston*: Noecker October, 1933.

This species is recognized as the conidial phase of *Pseudovalsa longipes*.

### 2. *Coryneum pustulatum* Pk.

Plate IV. Figs. 68-70

Acervulus shield-shaped, flattened, erumpent, through a slightly broken fissure; spores long, subclavate or subfusiform, brown, 6-7 septate, often curved, 62-75 x 10-12 $\mu$ , sporophores short.

On *Castanea dentata* (Marsh.) Borkh. *Creston*: Noecker August, 1933; *Atlantic*: Butler September, 1933.

This species is very like *Coryneum kunzei* from oak, differing chiefly in its slightly narrower spores and shorter conidiophores. Possibly this species is the conidial stage of *Melanconis modonia* Tul. which has a *Coryneum* associated with it. Another specimen collected by Butler from this same locality (*Atlantic*) showed the two together.

### 3. *Coryneum juniperinum* Ell.

Plate IV. Figs. 71-73

Acervuli erumpent, forming dark brown, hemispherical, subconfluent, tufts along the mid-vein of the living needles. Spores vermiform-cylindrical, dark brown 6-8 septate, 35-40 x 6-8 $\mu$  on short stout sporophores.

On *Juniperus communis* L. *Decorah*: Holway, May, 1882.

### Excluded Species

*Diaporthe stictostoma* (Ell.) Sacc. on *Amelanchier canadensis* (4) is a *Cryptosporella*. (p. 267).

*Diaporthe carpinicola* Fekl. on *Carpinus* sp. (Holway 1882) is *Melanconis hyperopta* (Nit.) Wehm. (p. 254).

*Diaporthe ostryae* Dearn. on *Ostrya virginiana* is *Melanconis ostryae* (Dearn.) Wehm. (21)



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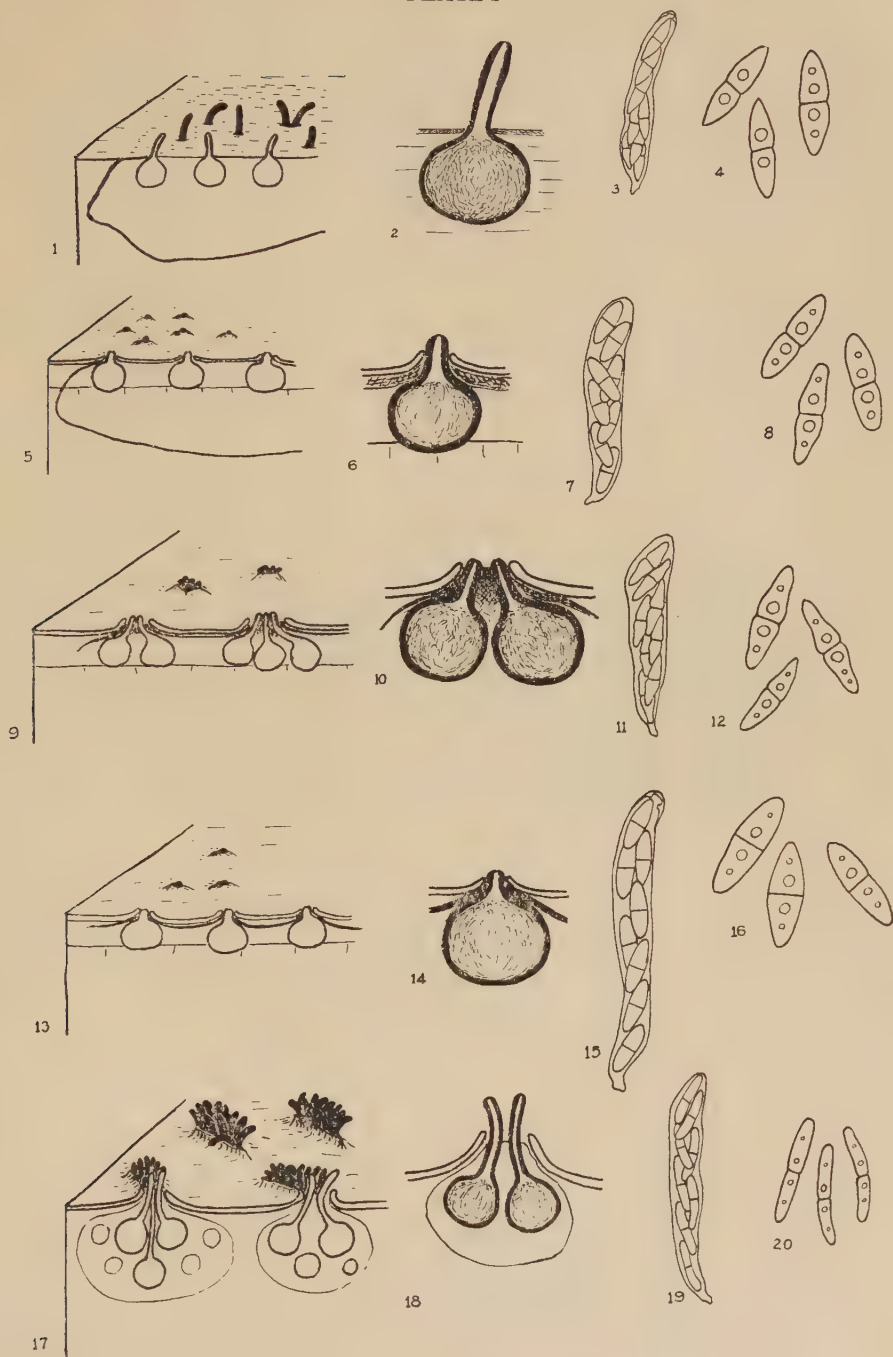
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## PLATE I

- Figs. 1- 4. *Diaporthe arctii*. 1. Habit (x10). 2. Perithecium (x50). 3. Ascus (x500). 4. Ascospores (x1000).
- Figs. 5- 8. *Diaporthe eres*. 5. Habit (x10). 6. Perithecium (x50). 7. Ascus (x500). 8. Ascospores (x100).
- Figs. 9-12. *Diaporthe spiculosa*. 9. Habit (x10). 10. Perithecia (x50). 11. Ascus (x500). 12. Ascospores (x1000).
- Figs. 13-16. *Diaporthe viburni*. 13. Habit (x10). 14. Perithecium (x50). 15. Ascus (x500). 16. Ascospores (x1000).
- Figs. 17-20. *Diaporthe prunicola*. 17. Habit (x10). 18. Perithecia (x50). 19. Ascus (x500). 20. Ascospores (x1000).

PLATE I





## PLATE II

- Figs. 21-24. *Diaporthe leiphaemia* var. *raveneliana*. 21. Habit (x10). 22. Perithecia (x50).
- Figs. 25-28. *Diaporthe decedens*. 25. Habit (x10). 26. Perithecium (x25). 27. Ascus (x500). 28. Ascospores (x1000).
- Figs. 29-34. *Diaporthe pruni*. 29. Habit (x10). 30. Perithecia (x25). 31. Ascus (x500). 32. Alpha conidia (x1000). 33. Beta conidia (x1000). 34. Ascospores (x1000).
- Figs. 35-38. *Diaporthe padi*. 35. Habit (x10). 36. Perithecia (x25). 37. Ascus (x500). 38. Ascospores (x1000).
- Figs. 39-42. *Diaporthe acerina*. 39. Habit (x10). 40. Perithecia (x25). 41. Ascus (x500). 42. Ascospores (x1000).

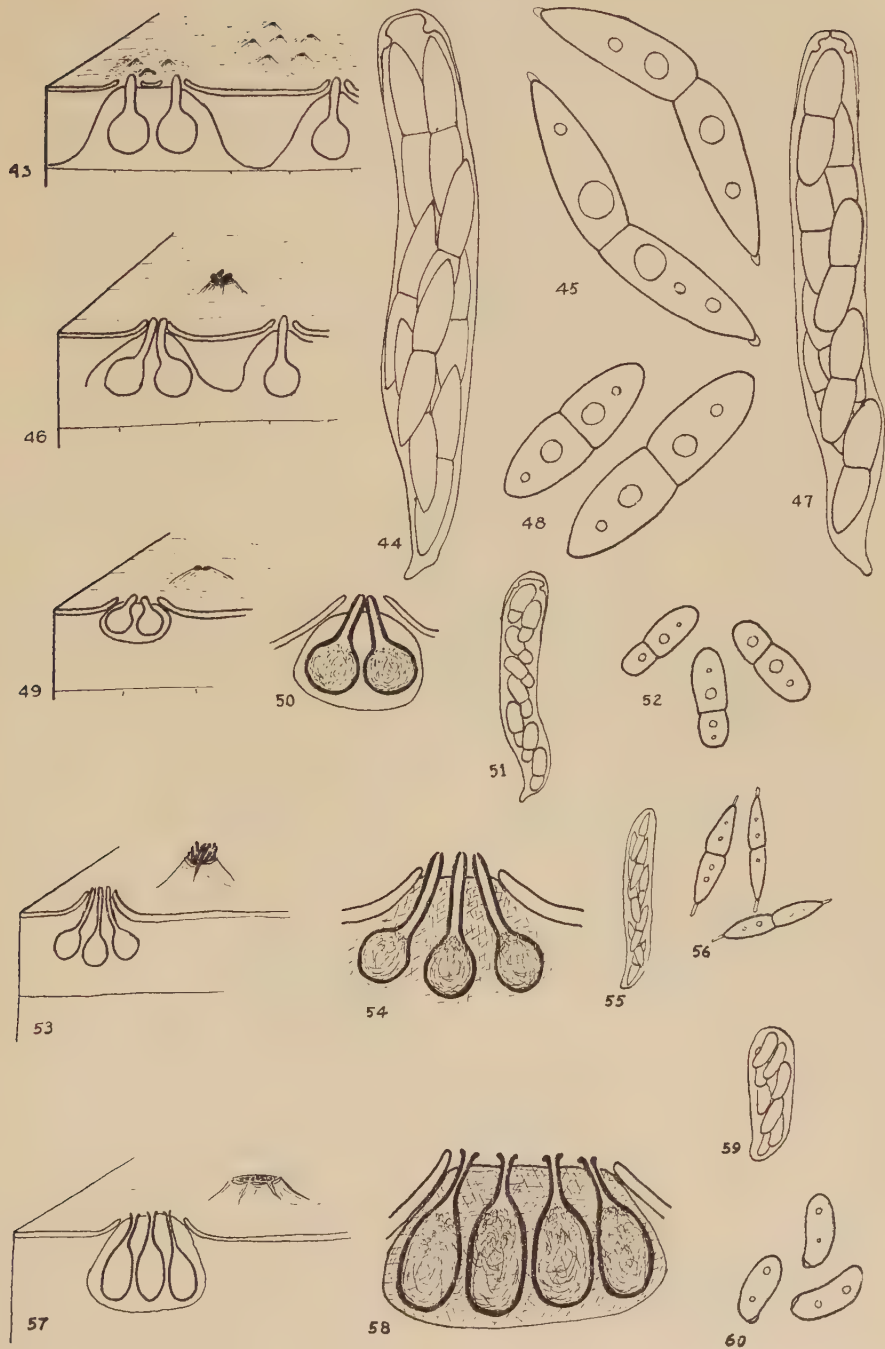
PLATE II



## PLATE III

- Figs. 43-45. *Diaporthe tesella*. 43. Habit (x10). 44. Ascus (x500). 45. Ascospores (x1000).
- Figs. 46-48. *Diaporthe tiliacea*. 46. Habit (x10). 47. Ascus (x500). 48. Ascospores (x1000).
- Figs. 49-52. *Apioportha apiospora*. 49. Habit (x10). 50. Perithecia (x25). 51. Ascus (x500). 52. Ascospores (x1000).
- Figs. 53-56. *Cryptodiaporthe aculeans*. 53. Habit (x10). 54. Perithecia (x25). 55. Ascus (x500). 56. Ascospores (x1000).
- Figs. 57-60. *Apioportha anomala*. 57. Habit (x10). 58. Perithecia (x25). 59. Ascus (x500). 60. Ascospores (x1000).

## PLATE III

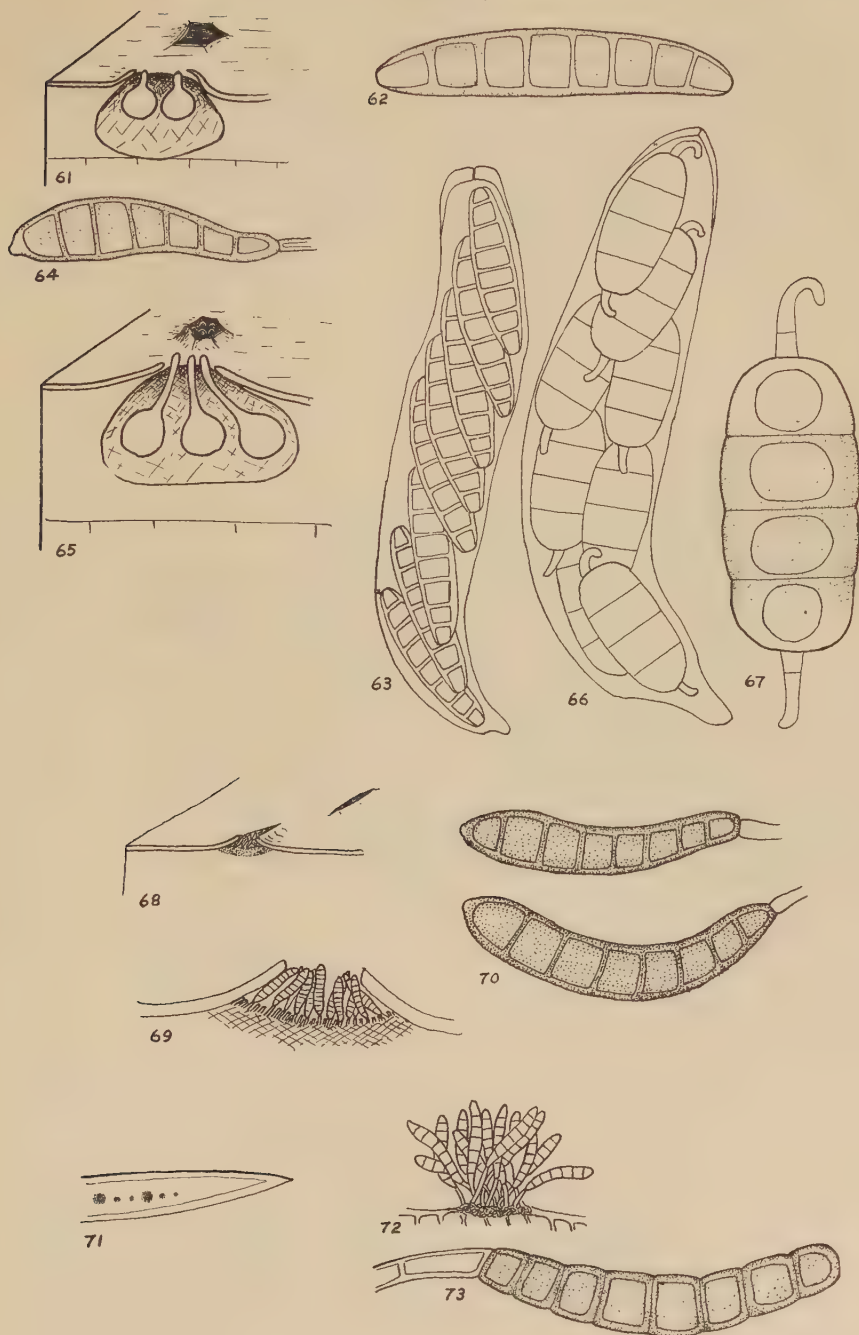




## PLATE IV

- Figs. 61-64. *Pseudovalsa longipes*. 61. Habit (x10). 62. Ascospore (x1000). 63. Ascus (x500). 64. Conidium (x1000).
- Figs. 65-67. *Pseudovalsa ulmi*. 65. Habit (x25). 66. Ascus (x500). 67. Ascospore (x1000).
- Figs. 68-70. *Coryneum pustulatum*. 68. Habit (x10). 69. Acervulus (x50). 70. Conidia (x1000).
- Figs. 71-73. *Coryneum juniperinum*. 71. Habit (x10). 72. Acervulus (x50). 73. Conidium (x1000).

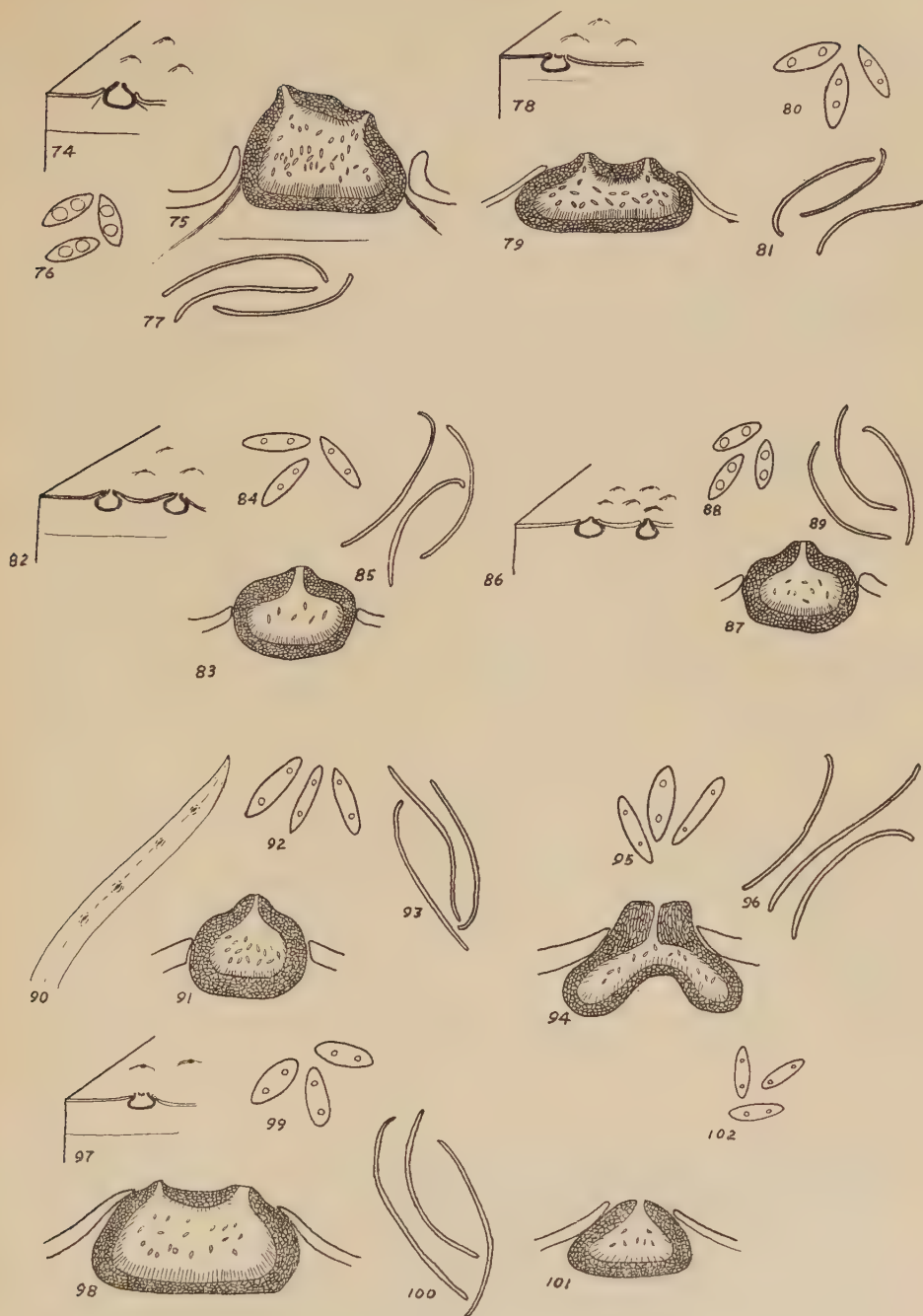
## PLATE IV



## PLATE V

- Figs. 74- 77. *Phomopsis acerina*. 74. Habit (x20). 75. Pycnidium (x50). 76. Alpha conidia (x1000). 77. Beta conidia (x1000).
- Figs. 78- 81. *Phomopsis subordinaria*. 78. Habit (x10). 79. Pycnidium (x500). 80. Alpha conidia (x1000). 81. Beta conidia (x1000).
- Figs. 82- 85. *Phomopsis stewartii*. 82. Habit (x10). 83. Pycnidium (x50). 84. Alpha conidia (x1000). 85. Beta conidia (x1000).
- Figs. 86- 89. *Phomopsis vexans*. 86. Habit (x10). 87. Pycnidium (x50). 88. Alpha conidia (x1000). 89. Beta conidia (x1000).
- Figs. 90- 93. *Phomopsis juniperovora*. 90. Habit (x5). 91. Pycnidium (x50). 92. Alpha conidia (x1000). 93. Beta conidia (x1000).
- Figs. 94- 96. *Phomopsis conorum*. 94. Pycnidium (x50). 95. Alpha conidia (x1000). 96. Beta conidia (x1000).
- Figs. 97.-100. *Phomopsis mali*. 97. Habit (x10). 98. Pycnidium (x50). 99. Alpha conidia (x1000). 100. Beta conidia (x1000).
- Figs. 101-102. *Phomopsis salicina*. 101. Pycnidium (x50). 102. Alpha conidia (x1000).

## PLATE V







# PRELIMINARY STUDIES ON THE COMPARATIVE VALUE OF SOME SPRAYS AND DUSTS IN POTATO INSECT CONTROL<sup>1</sup>

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Received October 21, 1939

The economic importance of potatoes as a truck crop in Iowa warrants an efficient and economical method of controlling potato insect pests. Bordeaux mixture in combination with paris green or other asenicals such as calcium arsenate, for many years has been recommended as the most efficient insecticide for general use in the control of potato insects. Since insecticidal dusts are more easily applied and require less expensive equipment for application both in gardens and in fields, it has been considered desirable to find a dust or combination of dusts which would give as effective control as does Bordeaux.

The most numerous insect pests of potatoes in Iowa are the following: potato leafhopper, *Empoasca fabae* (Harris); Colorado potato beetle, *Leptinotarsa decemlineata* (Say); potato flea beetle, *Epitrix cucumeris* (Harris); and aphids, mainly *Myzus persicae* (Sulz.).

During the summer of 1938, spraying and dusting experiments were conducted at Ames, Iowa, on the Experiment Station plots. Only the potato leafhopper was considered abundant enough throughout the season to affect the results obtained in this experiment.

## GENERAL PLAN OF THE EXPERIMENT

Experimental plots were arranged in a 7 x 7 Latin square 210 feet long by 70 feet wide and planted to certified Rural Russet potatoes treated with Semesan Bel for the control of seed-borne *Rhizoctonia* and potato scab and for protection of the seeds against rotting. Each plot in the Latin square was 30 feet long by 10 feet wide and contained three rows 3 feet apart. The hills within the rows were spaced 14 inches apart. Alleys between plots were 4 feet wide. The treatments were: sulphur-paris green dust; Bordeaux-paris green spray; sulfur dust; lime spray; derris-talc dust; paris green spray; paris green-talc dust.

## TREATMENT OF PLOTS

The Bordeaux spray was used as a standard in this experiment, since Bordeaux mixture (4-4-50), to which has been added paris green, is generally recommended for the control of potato insects in Iowa. The spray consists of 4 pounds of copper sulfate and 4 pounds of high calcium

<sup>1</sup> Journal Paper No. J-679 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 141.

<sup>2</sup> The authors are deeply indebted to Dr. C. J. Drake, Professor G. W. Snedecor, Miss Gertrude Cox, and Dr. C. H. Richardson for many helpful suggestions and criticisms.

hydrated lime in 50 gallons of water plus 1 pound of paris green per 50 gallons of spray.

The lime spray consisted of 6 pounds of high calcium hydrated lime in 50 gallons of water; the paris green spray of 1 pounds of paris green to 2 pounds of high calcium hydrated lime in 50 gallons of water. All spray applications were made with a 10-gallon Paragon hand sprayer equipped with a single B-14 brass nozzle for mist spraying. The spray was applied to the under side of the leaves at 120 to 150 pounds of pressure at the rate of approximately 100 gallons of spray per acre.

The sulfur-paris green dust consisted of 10 pounds of 300-mesh dusting sulfur to 1 pound of paris green; the sulfur dust of the sulfur alone; the derris-talc dust of 1 pound of 300-mesh derris (4 per cent) to 2 pounds of talc; and the paris green-talc dust of 1 pound of paris green to 10 pounds of talc. All dusts were applied with a Root Challenge hand duster of 6 pounds capacity equipped with two nozzles. The dusts were applied at the rate of approximately 60 pounds to the acre per application.

Each plot received four applications of the spray or dust. The first application was made July 8 and the process repeated at intervals of 10 to 12 days. The last application was made August 12. The insecticides were always applied on the same day and care was taken to prevent drifting of the dust or spray by use of muslin screens. Dusting was done from 4:00 to 8:00 A. M. in the mornings on still days and while there was a considerable amount of dew on the plants. Spraying was done during the remainder of the same day. To eliminate differences in yield caused by damage by the Colorado potato beetle all plots were sprayed on July 2 with paris green-lime spray.

#### METHODS

In order to determine the effectiveness of each spray or dust application, counts were made of potato leafhopper nymphs and adults and the amount of "hopperburn" was estimated. Adult and nymphal counts were made throughout the season on the same three plants evenly spaced in each row. On each plant five leaflets were selected at random at the time of the count and used for each determination. Counts were made the day before the application of spray or dust and again from 2 to 3 days after each application depending upon weather conditions.

Estimation of damage was made on the same plants used for determining populations. Since the late stages of "hopperburn" are not easily distinguished from damage caused by arsenicals no attempt was made to differentiate between them. Five leaflets were selected as in the population counts and the percentage of "hopperburn" on each leaflet estimated. The determination of damage was made 4 to 8 days after treatments.

In addition to these data, tuber yields from the different plots were taken at the end of the season, since yields give the best estimate of the relative value of the different treatments. The mean yields are recorded in table 1; the statistical analysis followed the methods of Snedecor (1938).

## RESULTS

A comparison of yields in table 1 indicates that there are considerable differences for the treatments. Analysis of variance shows that these differences are highly significant.

TABLE 1. *Yields in pounds of tubers per plot*

| Plot       | Sulfur-paris green dust | Bordeaux-paris green spray | Sulfur dust | Lime spray | Derris-talc dust | Paris green spray | Paris green-talc dust |
|------------|-------------------------|----------------------------|-------------|------------|------------------|-------------------|-----------------------|
| 1.....     | 78.0                    | 91.0                       | 102.5       | 71.0       | 90.0             | 84.5              | 84.5                  |
| 2.....     | 105.5                   | 104.0                      | 142.0       | 90.5       | 135.5            | 96.5              | 90.0                  |
| 3.....     | 140.0                   | 150.0                      | 180.0       | 136.0      | 134.0            | 117.5             | 116.5                 |
| 4.....     | 165.0                   | 173.5                      | 146.0       | 136.5      | 148.0            | 145.5             | 126.0                 |
| 5.....     | 156.0                   | 188.0                      | 178.0       | 154.5      | 157.0            | 158.5             | 113.5                 |
| 6.....     | 158.5                   | 189.0                      | 190.0       | 144.5      | 150.5            | 157.0             | 104.5                 |
| 7.....     | 146.0                   | 157.5                      | 176.0       | 140.0      | 147.0            | 139.0             | 100.0                 |
| Mean ..... | 135.6                   | 150.4                      | 157.8       | 124.7      | 137.4            | 128.4             | 105.0                 |

The mean differences between treatments, with one exception, fall into three groups: Bordeaux-paris green spray and sulfur dust giving highest yields; sulfur-paris green dust, lime spray, derris-talc dust and paris green spray intermediate; and paris green-talc dust the lowest yields.

TABLE 2. *The analysis of variance of data in table 1*

| Source of variation | Degrees of freedom | Sum of squares | Mean square |
|---------------------|--------------------|----------------|-------------|
| Rows .....          | 6                  | 606.5          | 101.08      |
| Columns .....       | 6                  | 30,315.4       | 5,052.57    |
| Treatments .....    | 6                  | 13,145.3       | 2,190.88    |
| Error .....         | 30                 | 5,470.5        | 182.35      |
| Total .....         | 48                 | 49,537.7       |             |

## DISCUSSION OF RESULTS

Fenton and Hartzell (1922 and 1923), De Long (1934), Menusan (1938), and Munro and Schifino (1938) have shown that Bordeaux mixture is a very effective spray for the control of the potato leafhopper. The yields obtained in this experiment, table 1, agree with their results and were only surpassed in yields by plots treated with sulfur dust. Figure 1 shows that the application of Bordeaux reduces the population of nymphs and adults and that the residual effect is greater than that of any of the other insecticides used. This is shown in the counts made before





Fig. 1. Comparison of sulfur-paris green dust, paris green-talc dust, and sulfur dust with Bordeaux mixture in reducing leafhopper populations during the summer of 1938.

the second and third sprays where the period between sprays was extended to 12 days. After the third application, the length of time between sprays was shortened to 10 days, and, as may be noted from the graph, the residual effect disappears. Figure 3 shows the percentage of "hopper-burn" to be quite low throughout the season.

The use of dusts containing sulfur gave (Fig. 1) a similar reduction in leafhopper population after application of the insecticide and in the amount of damage (Fig. 3) throughout the season. The residual effect, however, was not as great as in the case of Bordeaux mixture. De Long (1934) and Menusan (1938) obtained similar results with sulfur. Roney

TABLE 3. Differences in mean yields in pounds between different treatments and the Bordeaux standard

| Treatment                        | Mean yield | Difference from Bordeaux mean yield |
|----------------------------------|------------|-------------------------------------|
| Bordeaux-paris green spray ..... | 150.4      |                                     |
| Sulfur-paris green dust .....    | 135.6      | 14.8                                |
| Sulfur dust .....                | 157.8      | 7.4                                 |
| Lime spray .....                 | 124.7      | 25.7                                |
| Derris-Talc dust .....           | 137.4      | 13.0                                |
| Paris green spray .....          | 128.4      | 22.0                                |
| Paris green-Talc dust .....      | 105.0      | 45.4                                |

Difference necessary for significance, 14.7 pounds per plot.

and Thomas (1935) found that sulfur dusts were highly effective in the control of the potato leafhopper on beans.

The addition of paris green to the sulfur decreases the leafhopper population more than sulfur alone but at the same time decreases the yield. This decrease in yield is probably owing to the increase in damage, part of which might be caused by burning from paris green.

Paris green applied as a dust with an inert carrier, such as talc, caused (Fig. 1) a pronounced reduction in leafhopper populations, after the second application, comparable to that obtained with sulfur-paris green dust. The amount of damage, however, is considerably greater. The large amount of damage on August 18 and 30 (Fig. 3) is definitely known to result partially from burning caused by the insecticide. This high amount of burning probably accounts for the decidedly lower plot yields (table 1). When paris green with hydrated lime is applied as a spray there is (Fig 2) little, if any, reduction of leafhoppers. This is reflected both in the amount of damage occurring (Fig. 3) and the reduction in yield.

Britton (1934) found that three applications of derris dust containing 0.4 per cent rotenone, used for Mexican bean beetle control, incidentally reduced the injury by the potato leafhopper, *Empoasca fabae*, on beans. The use of 2 per cent derris with talc as a carrier in this experiment shows (Fig. 2) that there was no consistency in the reduction of populations obtained with this dust. The damage (Fig. 3) was relatively high throughout the season as might be expected with a high population count. No explanation can be given here as to the reason for a high yield on these plots. Further work with this insecticide may give some explanation of these results.

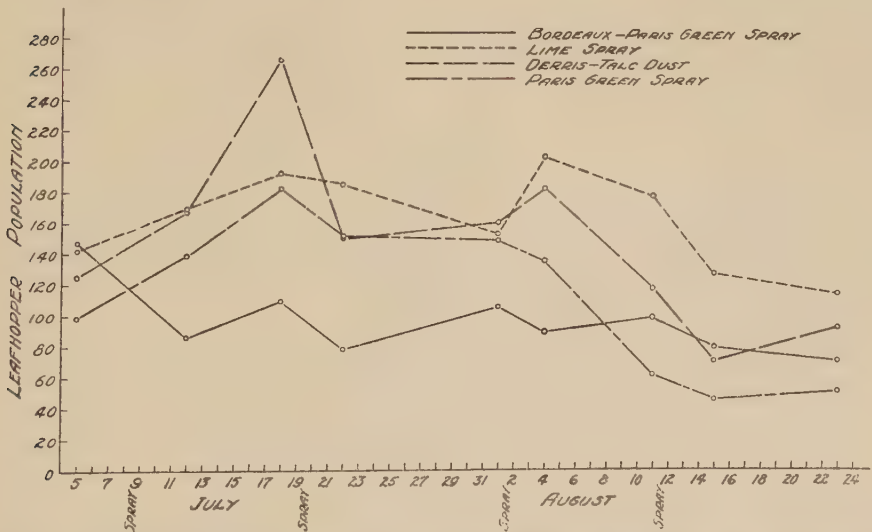


Fig. 2. Comparison of lime spray, derris-talc dust, and paris green spray with Bordeaux mixture in reducing leafhopper populations during the summer of 1938.

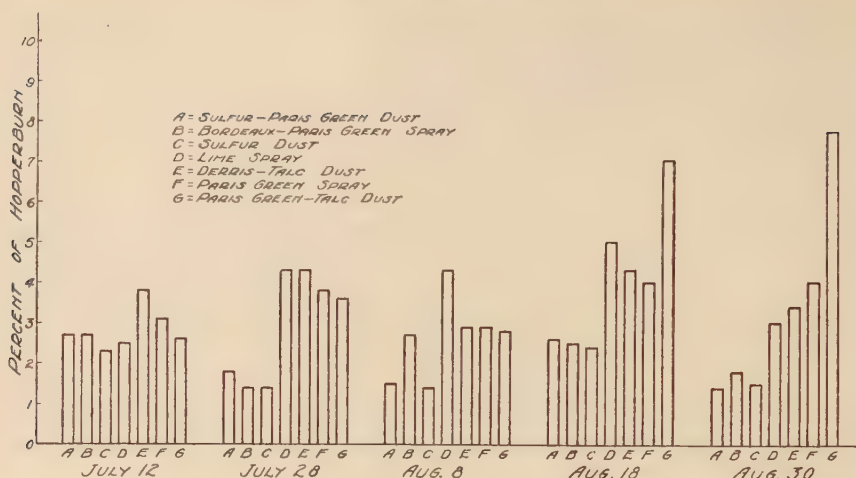


Fig. 3. A comparison of the percentage of "hopperburn" occurring at intervals throughout the season on plots treated with seven different insecticides.

In 1935 and 1936 McDaniel (1936, 1937) conducted field experiments on dahlia, potato, and alfalfa in which she used flour, talc, infusorial earth, and lime as dusts. She found that the potato leafhopper was repelled by these dusts owing to the white coating on the foliage.

In this experiment the authors used a high calcium hydrated lime spray which adhered well to the plants and resulted in a white coating. The data obtained on population and damage are quite similar to those obtained with derris as can be seen in figures 2 and 3. The yield from these plots, however, does not differ significantly from that obtained with derris, yet there is a reduction which might be considered due to a lessened degree of control.

#### SUMMARY

1. Sulfur dust (325 mesh) and 4-4-50 Bordeaux mixture were effective in reducing the leafhopper population and the amount of damage due to "hopperburn." The use of these two insecticides resulted in a significantly increased yield over all the other plots with the exception of the yield obtained on derris-treated plots.

2. Sulfur dust containing paris green was as effective as sulfur dust and 4-4-50 Bordeaux mixture in reducing leafhopper populations and the amount of damage, but the yield was lowered significantly possibly because of some harmful action of paris green on the plant.

3. Counts on plots treated with paris green applied as a dust, with talc as the inert carrier, show a pronounced reduction in leafhopper populations comparable to that obtained with sulfur-paris green dust. The amount of damage was higher than on any of the other plots partially because of burning caused by the insecticide. The yield on these plots was significantly lower than any of the others.

4. Counts on plots sprayed with paris green-hydrated lime show little, if any, reduction in leafhopper populations. Damage is high and there is a considerable reduction in yield .

5. The derris-talc dust is not consistent in its reduction of leafhopper populations. Damage was relatively high but the yield also was high.

6. Plots treated with a high calcium hydrated lime spray gave somewhat similar results to those obtained on derris-treated plots with a slightly, but not significantly, lower mean yield.

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# EXAMINATION OF ICE CREAM WITH THE BURRI SMEAR CULTURE TECHNIC<sup>1</sup>

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Received October 26, 1939

The Burri smear culture technic has been used primarily for the determination of numbers of bacteria in milk (1, 2). It also has been adapted to the examination of butter (4). With this solid dairy product it can be used to determine the distribution of organisms as well as the general numbers present, and in some instances species not developing on poured plates have been isolated with it.

Ice cream is another dairy product in which the distribution of organisms apparently is of significance. Excluding organisms coming from the air, there can be a variation in the distribution because of irregular contamination during manufacture and also as a result of dipping with contaminated equipment. The Burri technic has been employed in the examination of ice cream, and representative data obtained with it are given herein.

## METHOD

In applying the Burri technic to ice cream small portions are picked with a sterile platinum needle under a low power binocular, and each is spread over the surface of a dry agar slope. The portions are intended to weigh 0.02 mg. and are picked from firm ice cream, the ice cream being surrounded with ice if necessary. The original surface can be used, a fresh surface can be exposed with a sterile instrument or the interior and surface can be compared. A binocular aids in keeping the portions fairly uniform in size and also in actually picking each portion rather than scraping it from a relatively large area, which is a point of importance in studying distribution of organisms.

Since the medium and incubation conditions that are standard for milk are widely used with ice cream, there ordinarily is an advantage in using them in applying the technic to ice cream. Occasionally other media and incubation conditions may be desirable, such as the addition of skim milk to the agar for detection of proteolytic types or the use of a higher temperature for detection of thermophiles.

Ordinarily 10 portions are picked from a sample but larger or smaller numbers may be used for more or less detailed results. With low count ice cream, two or even more portions can be smeared over the same agar surface.

The numbers of colonies developing from the various portions indi-

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<sup>1</sup> Journal Paper No. J-691 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 119.

TABLE 1. Numbers of colonies on slopes (Burri technic) made with package ice cream having plate counts under 100,000 per ml.\*  
About 0.02 mg. ice cream per slope

| Slope number | Sample number |   |   |   |   |    |    |    |   |    |    |    |    |    |    |
|--------------|---------------|---|---|---|---|----|----|----|---|----|----|----|----|----|----|
|              | 1             | 2 | 3 | 4 | 5 | 6  | 7  | 8  | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1            | 1             | 0 | 1 | 0 | 0 | 2  | 1  | 2  | 0 | 0  | 0  | 3  | 0  | 1  | 0  |
| 2            | 0             | 0 | 1 | 0 | 0 | 0  | 1  | 0  | 0 | 0  | 0  | 1  | 3  | 0  | 0  |
| 3            | 3             | 0 | 0 | 0 | 2 | 4  | 4  | 2  | 0 | 0  | 0  | 0  | 1  | 1  | 1  |
| 4            | 1             | 0 | 1 | 0 | 0 | 4  | 3  | 2  | 0 | 0  | 0  | 0  | 0  | 0  | 0  |
| 5            | 1             | 0 | 0 | 1 | 1 | 0  | 1  | 0  | 1 | 0  | 0  | 0  | 0  | 0  | 0  |
| 6            | 0             | 0 | 2 | 1 | 0 | 3  | 6  | 0  | 0 | 0  | 0  | 0  | 1  | 1  | 1  |
| 7            | 0             | 0 | 0 | 0 | 0 | 3  | 4  | 2  | 1 | 0  | 0  | 0  | 1  | 2  | 1  |
| 8            | 0             | 0 | 0 | 0 | 0 | 2  | 1  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 1  |
| 9            | 0             | 0 | 0 | 0 | 0 | 5  | 10 | 1  | 1 | 0  | 0  | 0  | 1  | 0  | 2  |
| 10           | 0             | 0 | 0 | 1 | 0 | 3  | 3  | 1  | 0 | 1  | 2  | 0  | 0  | 4  | 2  |
| Total        | 6             | 0 | 5 | 3 | 3 | 26 | 34 | 10 | 3 | 1  | 2  | 4  | 7  | 12 | 8  |

\* In sampling package ice cream for either the Burri or plate technic the surface was always discarded.

cate the distribution and general bacterial content. With the usual samples the number per slope are low and counting is relatively easy. The maximum number of colonies that can be counted readily on a slope is about 100, depending largely on their size and the tendency to grow together. Numbers in excess of 100 often can be estimated satisfactorily, but under these conditions many organisms undoubtedly fail to grow. The smears can be examined before the end of the usual incubation period, and if the numbers of colonies are excessive they are counted. The average number of colonies per smear can be transposed to a gram basis if desired.

#### EXPERIMENTAL WEIGHTS OF PORTIONS OF ICE CREAM PICKED

The weights of portions of ice cream picked were determined with a microbalance, using vanilla and chocolate ice cream. Loss of water during the weighing was negligible. In 47 trials made over a period of about 6 months, the weights ranged from 0.010 to 0.036 mg. (with only two below 0.012 mg. and only one above 0.030 mg.) and averaged 0.017 mg.; about 53 per cent of the samples were within the limits of 0.015 and 0.025 mg. The variations were much greater than those encountered in attempting to pick 0.05 mg. of butter. With soft ice cream there was a definite tendency to obtain relatively large amounts; undoubtedly other factors, such as differences in overrun, structure, etc. of the ice cream and also the experience of the person using the technic, contribute to variations in the weights.

#### PACKAGE ICE CREAM

The numbers of colonies on slopes smeared with the interior of package ice cream are given in tables 1 and 2. The data on samples having plate counts below 100,000 per ml. are presented in table 1. Many of the slopes were sterile and on those showing growth the numbers of colonies were never large, the maximum being 10. In general, the various slopes from a sample showed relatively little variation in numbers of colonies, which indicates a rather uniform distribution of organisms. The greatest variations occurred with samples 6 and 7, which are the ones yielding the highest totals for the 10 slopes.

Table 2 deals with samples having plate counts above 100,000 per ml. Few slopes were sterile and a considerable percentage had large numbers of colonies, the maximum being 610. While the different slopes from a sample sometimes showed little variation in numbers of colonies, in other cases they showed considerable variation, which suggests an irregular distribution of the bacteria.

#### DIPPED ICE CREAM

Tables 3 and 4 present the numbers of colonies on slopes smeared with the interior and surface of dipped ice cream. The data on samples having plate counts (on interior) below 100,000 per ml. are given in table 3. Slopes from the interior often were sterile and on those showing growth



TABLE 2. Numbers of colonies on slopes (Burri technic) made with package ice cream having plate counts over 100,000 per ml.\*  
About 0.02 mg. ice cream per slope

| Slope number | Sample number |     |    |     |    |     |      |     |    |    |      |      |     |     |     |
|--------------|---------------|-----|----|-----|----|-----|------|-----|----|----|------|------|-----|-----|-----|
|              | 16            | 17  | 18 | 19  | 20 | 21  | 22   | 23  | 24 | 25 | 26   | 27   | 28  | 29  | 30  |
| 1            | 2             | 7   | 8  | 8   | 2  | 13  | 224  | 14  | 7  | 2  | 380  | 220  | 2   | 28  | 9   |
| 2            | 3             | 7   | 2  | 8   | 3  | 45  | 0    | 16  | 5  | 7  | 260  | 400  | 0   | 60  | 12  |
| 3            | 2             | 19  | 9  | 20  | 7  | 10  | 230  | 23  | 3  | 4  | 180  | 450  | 1   | 64  | 46  |
| 4            | 4             | 2   | 3  | 4   | 4  | 26  | 220  | 13  | 1  | 4  | 320  | 180  | 210 | 6   | 8   |
| 5            | 2             | 13  | 5  | 7   | 6  | 150 | 120  | 13  | 4  | 6  | 250  | 180  | 0   | 42  | 23  |
| 6            | 6             | 45  | 5  | 6   | 0  | 18  | 150  | 18  | 2  | 5  | 530  | 520  | 6   | 90  | 12  |
| 7            | 2             | 16  | 7  | 9   | 20 | 15  | 200  | 11  | 3  | 7  | 500  | 260  | 5   | 64  | 16  |
| 8            | 4             | 1   | 4  | 24  | 7  | 13  | 210  | 11  | 1  | 5  | 160  | 610  | 6   | 20  | 7   |
| 9            | 1             | 9   | 7  | 3   | 6  | 22  | 220  | 31  | 0  | 3  | 480  | 24   | 6   | 50  | 9   |
| 10           | 2             | 4   | 9  | 21  | 1  | 17  | 130  | 24  | 6  | 2  | 360  | 500  | 5   | 100 | 11  |
| Total        | 28            | 123 | 59 | 110 | 56 | 329 | 1704 | 174 | 32 | 45 | 3420 | 3344 | 241 | 524 | 153 |

\* In sampling package ice cream for either the Burri or plate technic the surface was always discarded.

TABLE 3. Numbers of colonies on slopes (Burri technic) made with interior and surface of dipped ice cream having plate counts (on interior) under 100,000 per ml.  
About 0.02 mg. ice cream per slope

| Slope<br>number | Sample number |       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-----------------|---------------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|                 | 31            |       | 32   |      | 33   |      | 34   |      | 35   |      | 36   |      | 37   |      | 38   |      |
|                 | int.*         | sur.* | int. | sur. | int. | sur. | int. | sur. | int. | sur. | int. | sur. | int. | sur. | int. | sur. |
| 1               | 1             | 1     | 1    | 1    | 1    | 20   | 1    | 4    | 1    | 0    | 0    | 1    | 2    | 0    | 0    | 1    |
| 2               | 5             | 1     | 0    | 0    | 0    | 6    | 3    | 3    | 0    | 0    | 0    | 0    | 0    | 7    | 1    | 0    |
| 3               | 4             | 0     | 1    | 1    | 0    | 9    | 1    | 3    | 0    | 0    | 0    | 0    | 0    | 1    | 0    | 1    |
| 4               | 2             | 1     | 0    | 0    | 2    | 1    | 1    | 2    | 0    | 0    | 1    | 2    | 2    | 1    | 1    | 1    |
| 5               | 1             | 1     | 0    | 2    | 0    | 12   | 3    | 4    | 1    | 0    | 0    | 0    | 0    | 20   | 0    | 1    |
| 6               | 3             | 0     | 3    | 2    | 1    | 17   | 1    | 5    | 0    | 0    | 0    | 0    | 1    | 1    | 0    | 1    |
| 7               | 3             | 0     | 0    | 1    | 0    | 92   | 0    | 1    | 1    | 0    | 1    | 0    | 5    | 1    | 1    | 1    |
| 8               | 1             | 1     | 0    | 2    | 0    | 13   | 4    | 3    | 1    | 0    | 0    | 0    | 0    | 1    | 0    | 1    |
| 9               | 4             | 1     | 0    | 1    | 0    | 12   | 1    | 1    | 0    | 0    | 0    | 0    | 2    | 2    | 1    | 0    |
| 10              | 0             | 1     | 0    | 2    | 1    | 9    | 2    | 1    | 0    | 0    | 0    | 0    | 3    | 1    | 0    | 0    |
| Total           | 24            | 7     | 5    | 12   | 5    | 191  | 17   | 27   | 4    | 0    | 2    | 3    | 15   | 35   | 4    | 7    |

\*int. = interior; sur. = surface



the numbers of colonies were small, with a maximum of 5. Usually the different slopes from a sample varied relatively little in numbers of colonies. Slopes from the surface were much the same as those from the interior, except with samples 33 and 37. With sample 33, the numbers of colonies on the slopes from the interior ranged from 0 to 2 and those on the slopes from the surface varied from 1 to 92; the surface evidently was contaminated heavily during the dipping. With sample 37, one of the slopes from the surface developed 20 colonies but otherwise the surface counts were much the same as the interior counts. In general the total counts for the 10 slopes were higher on the surface than on the interior, the two exceptions being samples 31 and 35.

Table 4 deals with dipped ice cream having plate counts (on interior) over 100,000 per ml. Few slopes from the interior were sterile and some had large numbers of colonies, the maximum being 107; with some samples the slopes showed little variation in numbers of colonies but with the majority the variations were large. Most slopes from the surface showed growth also and some had extremely large numbers of colonies with a maximum of 940. Commonly, the different slopes from a sample showed considerable variation in numbers of colonies. With all the samples, total counts on the 10 slopes from the interior were lower than on those from the surface and with some (e. g. 42, 43 and 44) the differences were striking, indicating a heavy contamination of the surface from the dipper.

Figures 1, 2 and 3 show representative slopes from the interior and surface of dipped ice cream and illustrate variations in the contamination of the surface.

#### COMPARISON OF ICE CREAM COUNTS BY THE BURRI TECHNIC AND PLATE METHOD

A comparison of counts on ice cream by the Burri technic (average number of colonies per slope  $\times$  50,000) and the plate method is given in table 5. Both counts represent only the interior of the ice cream. The first 50 samples are those on which data were obtained for the previous tables.

As would be expected, there was no close agreement between the Burri and plate counts. In general, the counts were higher with the Burri technic although in some cases they were lower. The differences sometimes were small, but in other cases they were much greater than could be accounted for by errors in the weights of ice cream picked. Since the plate count does not accurately determine the numbers of organisms, any technic differing from it would not be likely to give the same results.

Although the Burri counts were often considerably higher than the plate counts, they indicated the general sanitary quality of the ice cream. With most of the samples, such information could have been obtained from a very few slopes.

#### DISCUSSION

The primary advantage in using the Burri technic in examination of ice cream appears to be the opportunity to study distribution of organ-



TABLE 5. *Comparison of ice cream counts by the Burri technic and the plate method*

| Sample number | Estimated* Burri count | Plate count | Sample number | Estimated* Burri count | Plate count |
|---------------|------------------------|-------------|---------------|------------------------|-------------|
| 1             | 30,000                 | 42,000      | 33            | 25,000                 | 9,200       |
| 2             | <5,000                 | 2,000       | 34            | 85,000                 | 99,000      |
| 3             | 25,000                 | 20,400      | 35            | 20,000                 | 3,400       |
| 4             | 15,000                 | 4,100       | 36            | 10,000                 | 2,900       |
| 5             | 15,000                 | 4,400       | 37            | 75,000                 | 46,000      |
| 6             | 130,000                | 72,000      | 38            | 20,000                 | 8,500       |
| 7             | 170,000                | 84,000      | 39            | 25,000                 | 6,900       |
| 8             | 50,000                 | 7,400       | 40            | 20,000                 | 29,000      |
| 9             | 15,000                 | 6,000       | 41            | 120,000                | 210,000     |
| 10            | 5,000                  | 5,800       | 42            | 1,400,000              | 6,600,000   |
| 11            | 10,000                 | 24,200      | 43            | 440,000                | 108,000     |
| 12            | 20,000                 | 28,000      | 44            | 640,000                | 2,800,000   |
| 13            | 35,000                 | 47,000      | 45            | 240,000                | 181,000     |
| 14            | 60,000                 | 9,000       | 46            | 70,000                 | 1,130,000   |
| 15            | 40,000                 | 10,600      | 47            | 110,000                | 189,000     |
| 16            | 140,000                | 188,000     | 48            | 110,000                | 670,000     |
| 17            | 620,000                | 380,000     | 49            | 210,000                | 144,000     |
| 18            | 300,000                | 151,000     | 50            | 360,000                | 214,000     |
| 19            | 550,000                | 200,000     | 51            | 10,000                 | 3,100       |
| 20            | 280,000                | 118,000     | 52            | <5,000                 | 12,100      |
| 21            | 1,700,000              | 222,000     | 53            | 150,000                | 43,000      |
| 22            | 8,500,000              | 7,300,000   | 54            | 95,000                 | 19,200      |
| 23            | 870,000                | 450,000     | 55            | 25,000                 | 10,000      |
| 24            | 160,000                | 133,000     | 56            | 20,000                 | 32,000      |
| 25            | 230,000                | 310,000     | 57            | 50,000                 | 27,000      |
| 26            | 17,000,000             | 4,600,000   | 58            | 10,000                 | 32,000      |
| 27            | 17,000,000             | 4,900,000   | 59            | 150,000                | 46,000      |
| 28            | 1,200,000              | 1,860,000   | 60            | 15,000                 | 400         |
| 29            | 2,600,000              | 2,240,000   | 61            | 50,000                 | 8,700       |
| 30            | 770,000                | 180,000     | 62            | 100,000                | 70,000      |
| 31            | 120,000                | 6,100       | 63            | 5,000                  | 6,200       |
| 32            | 25,000                 | 21,000      | 64            | 270,000                | 60,000      |

\* Adjusted to an approximate value.

(Table 5 Continued)

| Sample number | Estimated* Burri count | Plate count | Sample number | Estimated* Burri count | Plate count |
|---------------|------------------------|-------------|---------------|------------------------|-------------|
| 65            | 20,000                 | 7,000       | 83            | 100,000                | 43,000      |
| 66            | 120,000                | 101,000     | 84            | 95,000                 | 30,000      |
| 67            | 150,000                | 115,000     | 85            | 25,000                 | 7,900       |
| 68            | 720,000                | 192,000     | 86            | 70,000                 | 36,000      |
| 69            | 1,800,000              | 270,000     | 87            | 1,400,000              | 92,000      |
| 70            | 2,900,000              | 560,000     | 88            | 25,000                 | 11,200      |
| 71            | 50,000                 | 5,500       | 89            | 5,000                  | 900         |
| 72            | 5,000                  | 3,700       | 90            | 85,000                 | 33,000      |
| 73            | 5,000                  | 4,000       | 91            | 35,000                 | 12,000      |
| 74            | 15,000                 | 3,000       | 92            | 5,000                  | 4,200       |
| 75            | 130,000                | 79,000      | 93            | 85,000                 | 28,000      |
| 76            | 10,000                 | 7,400       | 94            | 150,000                | 41,000      |
| 77            | 120,000                | 34,000      | 95            | 15,000                 | 2,900       |
| 78            | 30,000                 | 12,000      | 96            | 200,000                | 42,000      |
| 79            | 15,000                 | 2,700       | 97            | 160,000                | 15,000      |
| 80            | 10,000                 | 3,500       | 98            | 190,000                | 44,000      |
| 81            | 100,000                | 116,000     | 99            | 4,000,000              | 1,110,000   |
| 82            | <5,000                 | 1,400       | 100           | 15,000                 | 4,600       |

\* Adjusted to an approximate value.

isms in the product. This distribution is of special significance with dipped ice cream since dipping may result in serious contamination of the surface from the dipper. Krog and Dougherty (3) found that ice cream taken with the vendor's scoop generally yielded higher plate counts than that taken with a sterile spoon, while Yale and Hickey (5) did not find significant differences in plate counts of samples taken by the two methods. With the Burri technic, dipper contamination can be investigated by comparing the numbers of organisms at the interior and surface. The distribution of organisms should also be considered with package ice cream because the contamination during manufacture may not be uniform. With the samples examined, the distribution of organisms was usually more uniform in low count ice cream than in high count ice cream.

The general relationship between the Burri and plate counts suggests that the bacteria in ice cream commonly find more favorable growth conditions in smears on agar than in poured plates. This is in contrast to the situation with butter (4), but various factors, including the high pasteurization exposures usually employed with an ice cream mix and the frequent use of butter culture, give the two products rather different

floras. The fact that counts on ice cream tend to be higher with the Burri technic than with the plate method should be recognized in control work.

If only very general information on the numbers of bacteria in ice cream is desired, it can be obtained with very few slopes. Under these conditions the slopes can be prepared with less effort than is required to plate the ice cream. Moreover, the materials needed to prepare slopes are more easily transported to plants than those required to prepare plates, which is an advantage in case the objectives are such that the samples cannot be taken to a laboratory.

#### SUMMARY

The Burri smear culture technic is readily adaptable to the examination of ice cream but the variation in the weights of the portions picked is greater than with butter. The method is useful in studying the distribution of bacteria in ice cream, particularly in comparing the numbers of organisms at the interior and surface of dipped ice cream. It also gives a general idea of the numbers of bacteria in the product and such information can be obtained from a relatively few slopes. Counts with the Burri technic tend to be higher than plate counts.

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#### PLATE I

Fig. 1. Slopes made from interior and surface of dipped ice cream.  
Colonies indicate negligible contamination of surface of ice cream.

PLATE I



Interior

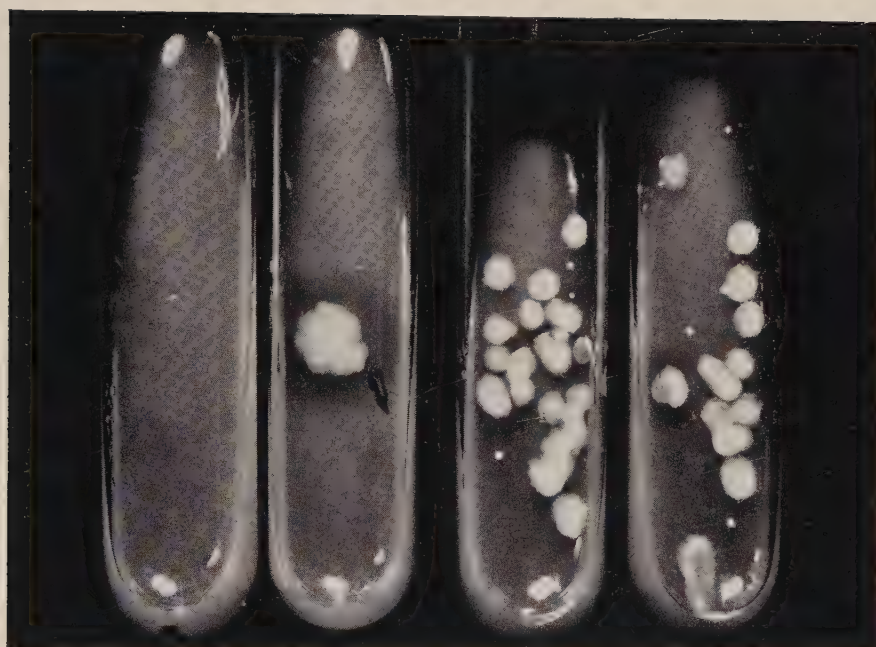
Surface



## PLATE II

Fig. 2. Slopes made from interior and surface of dipped ice cream.  
Colonies indicate considerable contamination of surface of ice cream.

PLATE II



Interior

Surface

## PLATE III

- Fig. 3. Slopes made from interior and surface of dipped ice cream.  
Colonies indicate heavy contamination of surface of ice cream with various types of organisms.

PLATE III



Interior

Surface





## PREPARATION OF AN ACTIVE JUICE FROM BACTERIA

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Received October 28, 1939

Our knowledge of bacterial metabolism has suffered for the want of a suitable method for the preparation of an active juice. A recent technique is that of Booth and Green (1938) employing a specially devised wet crushing mill. They have reported active bacterial juices attacking hexosediphosphate and simpler substrates. It is our purpose to describe a technique for the preparation of a bacterial juice obtained without the application of a mill. The method employs equipment which is readily available and is based on the use of powdered glass in the proper proportions for the disruption of the cells by grinding. Powdered glass has been employed previously by Stevens and West (1922) in the preparation of cell-free lipase solutions from a streptococcus.

### EXPERIMENTAL

#### MATERIAL

*Cells.* The cells generally employed were prepared from *Aerobacter indologenes* (23B). They were grown in 5-liter quantities of nutrient broth containing 1.0 per cent glucose, 0.3 per cent Bacto-peptone, 0.3 per cent  $(\text{NH}_4)_2\text{SO}_4$ , 0.5 per cent  $\text{K}_2\text{HPO}_4$  and 10 per cent tap water. The inoculated broth was incubated at 30° C. for 24 hours and the cells then recovered by centrifugation in a Sharples supercentrifuge at about 35,000 rpm. The yield from 5 liters of broth varied from 9 to 12 grams of cell paste which was employed without further washing.

*Glass.* Pyrex glass which had been cleaned and dried, was ground for 24 hours in a ball mill and sifted through a 30-mesh wire screen. The particle size varied for the most part from 2 to 5 microns in diameter. A dust mask should be worn by the experimenter when working with the dry powdered glass.

*Buffers.* All phosphate buffers employed, unless otherwise stated, were fifteenth molar.

#### PROCEDURE

Three grams of bacterial paste, 25 grams of ground glass and 7 ml. phosphate buffer (pH 7.0) were mixed intimately by means of a spatula. The resulting glass-cell paste resembled a rather firm batter. Ten grams of this mixture were then ground vigorously in a well-iced 4.5-inch mortar for 5 minutes. All such 10-gram portions were combined after grinding and extracted by thorough mixing with 2 ml. phosphate buffer (pH 6.6) for each 10-gram ground portion. The mixture was centrifuged for 4 minutes on a number 2 International Centrifuge at about 2,500 rpm.; the

supernate was then further clarified by a 2 minute centrifugation in a simple Beams<sup>1</sup> ultracentrifuge at about 175,000 rpm. The rotor of this centrifuge has a diameter of 1.25 inches. The supernate thus obtained was employed as the active juice. It was slightly brown and opalescent. A microscopic examination of a heavy smear of this juice showed large numbers of broken cell particles but only an occasional intact cell.

Such juices showed varying activities when tested in simple Warburg manometers in the presence of the following:

0.8 ml. juice  
0.0077 M sodium hexosediphosphate  
0.0137 M glucose  
0.0294 M NaHCO<sub>3</sub>  
1.7 ml. total volume

Atmosphere: CO<sub>2</sub>

Temperature: 30° C.

Table 1 indicates the activity of a number of juices prepared under similar conditions at different times. The gas evolved was calculated as CO<sub>2</sub>; no hydrogen was evolved.

TABLE 1. Activities of (*Aerobacter indologenes*) juices

| Juice number   | 1  | 2  | 3  | 4   | 5   | 6   | 7   |
|--|----|----|----|-----|-----|-----|-----|
| mm <sup>3</sup> gas evolved in 1 hour from substrate | 78 | 47 | 95 | 218 | 119 | 124 | 146 |
| Endogenous activity in 1 hour mm <sup>3</sup>        | —  | —  | 6  | 6   | 3   | 7   | 5   |

As shown later, the activity of the above juices may be greatly enhanced by the presence of boiled yeast extract.

The same juices when tested on the same substrates in a phosphate buffer under an atmosphere of nitrogen exhibit a lag as long as 90 minutes before any evolution of gas occurs. The difference in rate of gas evolution in the presence of phosphate buffers under an atmosphere of nitrogen and of bicarbonate buffers under an atmosphere of carbon dioxide is shown graphically in figure 1. Apparently the juice immediately attacks the substrate with the production of acids but the development of gaseous products requires an induction period.

The presence of boiled yeast juice may stimulate (table 2) the activity of the juices (nos. 2 and 3). The yeast juices very likely act by supplying coenzymes which may be considerably diluted in the preparation of the active bacterial juices. Previous experiments have shown that in the absence of added yeast juice no appreciable activity of bacterial juices in the presence of glucose was observed. It may be, however, that the effects of yeast juice in the latter case (i. e. stimulation of the glucose breakdown) may be due to the presence of hexosediphosphate extracted from the brewers' yeast; for data in the same table indicate that the evo-

<sup>1</sup> Appreciation is expressed to Dr. Alfred M. Lucas for the use of a Beams centrifuge.

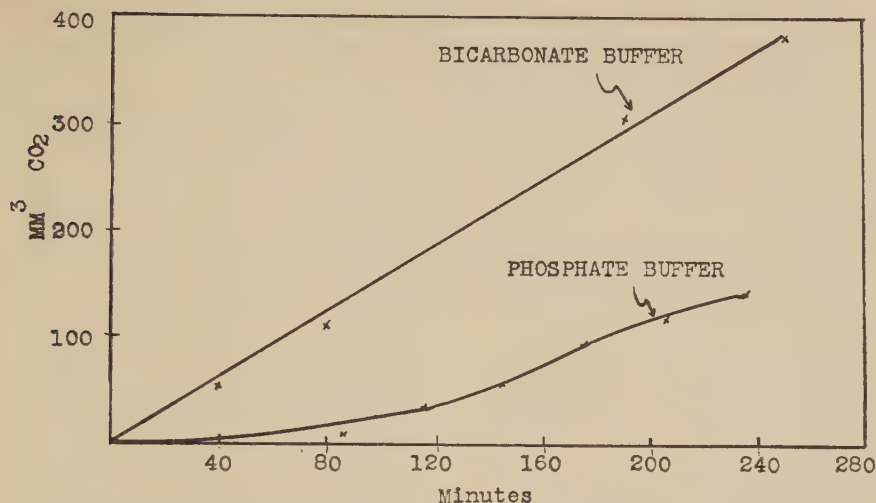


Fig. 1. Comparison of activity on phosphate and bicarbonate buffers.

lution of gas from mixtures of hexosediphosphate and glucose is greater than that which may be expected from a simple additive effect of the juice acting on the combined substrates. For example (juice no. 3) a total of  $89 + 65$  or  $154 \text{ mm}^3$  of gas per hour were evolved from glucose and hexosediphosphate when tested separately; yet from a mixture of the two substrates  $233 \text{ mm}^3$  of gas were evolved in the same time.

TABLE 2. Effects of glucose, hexosediphosphate and yeast juice on the activity of *Aerobacter indologenes* juices

|                   |    |          |          |          |        |         |
|-------------------|----|----------|----------|----------|--------|---------|
| Glucose           | —  | 0.0137 M | —        | —        | 0.0091 | 0.0091  |
| Hexosediphosphate | —  |          | 0.0077 M | 0.0077 M | 0.0051 | 0.0051  |
| Yeast juice       | —  | 0.1 ml.  | —        | 0.1 ml.  | —      | 0.1 ml. |
| Juice no. 1       | 5  | 16       | 36       | 36       | 91     | —       |
| " " 1a            | 5  | 18       | 37       | 38       | 92     | —       |
| " " 2             | 11 | 32       | 31       | 53       | 102    | 156     |
| " " 3             | 11 | 89       | 51       | 65       | 211    | 233     |

Values in  $\text{mm}^3$  gas evolved in one hour.

Bacterial juice: 0.8 ml.

Buffer: 0.029 M  $\text{NaHCO}_3$ , atmosphere  $\text{CO}_2$ , temperature  $30^\circ \text{C}$ ., volume 1.7 ml.

Yeast juice = supernate from boiled suspension of 1 gm. dried brewers' yeast + 10 ml. water. Time = 10 minutes.

In table 2, juices no. 1 and no. 1a were identical; the experiment was set up in duplicate to determine the reproducibility of results from the same juice. The data show that the results are duplicable. Juices no. 2 and no. 3 were prepared under identical conditions except that they were



ground separately, one immediately following the other; the same bacterial cells, glass and buffers were used, the grinding time was 5 minutes in both cases. The data show immediately that the activity of juices ob-

TABLE 3. *Reduction of methylene blue by juice of Aerobacter indologenes*

| Substrate        | Final concentration (molar) | Reduction time (minutes) |
|------------------|-----------------------------|--------------------------|
| None             | 0.0140                      | 390                      |
| Galactose        | 0.0138                      | 5                        |
| Mannitol         | 0.0125                      | 360                      |
| Sodium formate   | 0.0125                      | 5                        |
| Sodium succinate | 0.017                       | 15                       |
| Sodium fumarate  | 0.0173                      | 30                       |
| Sodium lactate   | 0.028                       | 30                       |
| Sodium pyruvate  | 0.0287                      | 75                       |
| Xylose           | 0.0167                      | 30                       |
| Dihydroxyacetone | 0.0278                      | 30                       |
| Erythritol       | 0.0225                      | 165                      |

tained from cells ground separately may vary considerably. For this reason, the cell extracts obtained after grinding should be mixed intimately before use; otherwise irregularities may arise.

A preliminary survey has shown the presence of dehydrogenases active on the substrates indicated in table 3 as determined by the Thunberg methylene blue technique.

Each Thunberg tube contained in addition to substrate, 1 ml. bacterial juice, 1 ml. phosphate buffer M/15 pH 7.6 and 1 ml. methylene blue  $1.2 \times 10^{-8} M$  (final pH approximately 7.2), atmosphere  $N_2$ , temperature  $30^\circ C.$ , total volume 4 ml.

To detect non-enzymatic reduction of methylene blue, controls containing boiled juice were run under the same conditions with each substrate. In no case did reduction occur in less than 390 minutes.

Several factors involved in the preparation of active juices have been investigated in some detail. A study of grinding time has shown that extended grinding progressively diminishes the activity of the cell-glass mixture and completely destroys it after 15 minutes. A determination of the optimum grinding time, judged by the activity of the juice in the respirometer, indicates that the most active juice is obtained after 4 minutes' grinding. Results are shown in table 4.

An effort was made to determine the effects of autolysis of the bacterial cell at  $13^\circ C.$  on the activity of the juice. Cells were autolyzed for varying periods up to and including 48 hours. At 48 hours less active juices were obtained. However, there seems to be little if any difference

TABLE 4. *Grinding time variations*

| Time in minutes | A  | B  | C  | D*    | Average |
|-----------------|----|----|----|-------|---------|
| 0               | 0  | 0  | 3  | 45    | 12.0    |
| 1               | 10 | 0  | 37 | 78    | 31.5    |
| 2               | —  | 31 | 49 | 105.2 | 48.8    |
| 3               | 28 | 74 | 50 | —     | 63.2    |
| 4               | 41 | 80 | 55 | 127.4 | 75.6    |
| 5               | 31 | 69 | 55 | 109.6 | 66.3    |

Activities of various samples of juice upon substrates of glucose and hexosediphosphate as given in mm<sup>3</sup> of CO<sub>2</sub> evolved per hour.

\* Mass of cells autolyzed 24 hours before grinding.

Conditions same as in table 1.

in the activity of the juices obtained up to a period of 36 hours. Juices from cells which have not been autolyzed show the same activities as those cells which have been autolyzed for this period. Our juices are now prepared directly from the cells without autolysis.

Efforts to determine an optimum pH for grinding and extraction have not met with marked success. The use of buffers varying between pH 6.0 and 8.0 in grinding resulted in little difference in the activity of the resulting juices as measured by CO<sub>2</sub> evolution. It is possible that the pH of the buffer used in extraction after grinding may be important. Our results, however, in using various phosphate buffers in extraction have been too variable to justify definite conclusions.

We have, however, determined the optimum concentration of the buffer used in extraction. It has been found that optimum results are obtained when 2 ml. of buffer are used to extract 10 grams of the ground cell-glass mixture. The use of more fluid results in less active juices while less does not yield a juice which is appreciably more active. A second extraction of the ground cell-glass mixture with 1 ml. buffer results in a juice which has but 20-25 per cent of the activity of the first extract (table 5).

TABLE 5. *Effect of volume of extracting fluid on activity of bacterial juice*

| ML. buffer used in extraction | Activity of 0.8 ml. resulting juice (mm <sup>3</sup> gas per hour) |                 |
|-------------------------------|--|-----------------|
|                               | 1st extraction   | *2nd extraction |
| 1                             | 149  | 56              |
| 2                             | 147  | 35              |
| 4                             | 72   | 20              |

\* After 1st extraction each residue was again extracted with 1 ml. buffer. Conditions same as in table 1.

Ground pyrex glass has been used throughout. In a single experiment, soft glass was employed and an active juice was obtained. However, if sufficient waste pyrex or other hard glass is available, it is to be preferred to soft glass for the reason that it is less likely to contribute soluble products to the cell extract.

The only means at hand for separation of viable cells from the juice is centrifugation. Passage of the juice through Seitz, Jena glass (3 auf 5) or Chamberland (numbers 3 and 7) filters completely inactivates it. Toluene in a concentration of 5 per cent reduces the activity of the juice by 80 to 90 per cent. All the viable cells cannot be eliminated by centrifugation because of difficulties of removing the juice from the centrifuge cup. Plate counts made from juice-substrate-buffer mixtures, taken from the Warburg flasks after testing, show generally from 250,000 to 750,000 viable cells per cup. However, the activity of the juice is not appreciably affected by that of the viable cells, for experiments with known numbers

TABLE 6. *Activity of known numbers of Aerobacter indologenes cells*

| Number of cells present (millions)                | 0  | 34 | 17 | 8.5 | 4.25 | 2.12 | 1.06 |
|---|----|----|----|-----|------|------|------|
| Activity in mm <sup>3</sup> CO <sub>2</sub> /hour | 10 | 65 | 28 | 21  | 6    | 9    | 7    |

Experimental conditions same as in table 1, except cell suspensions employed instead of cell juices.

of cells show that the presence of at least  $8.5 \times 10^6$  organisms per Warburg flask are necessary to produce an evolution of 21 mm<sup>3</sup> of gas per hour (table 6).

An experiment was undertaken in which known numbers of cells were added to an active juice to determine the number of viable cells necessary to influence the activity of the juice. The results (table 7) indicate that at least 15,000,000 viable cells per cup must be present to enhance to a detectable extent the activity of the juice. The conclusion may be drawn that the presence of 250,000 to 750,000 cells in the Warburg flasks did not appreciably influence the activity of the juices during the first 5 or 6 hours of the experiments. Since no experiments extended beyond that period of time it may be concluded that viable cells did not play an appreciable part in any of the experiments. Viable cells were determined by plate counts.

TABLE 7. *Effect of addition of known numbers of cells to juice of Aerobacter indologenes*

| Cells added (millions)                                   | 0  | 0.8 | 3.5 | 7.0 | 15.0 | 30.0 | 120.0 |
|--|----|-----|-----|-----|------|------|-------|
| Activity in mm <sup>3</sup> in 15 minutes after 1½ hours | 19 | 19  | 17  | 18  | 17   | 23   | 97    |

0.8 ml. bacterial juice in each cup. Experimental conditions same as in table 1.

A sample of the juice was divided into several portions and stored in a frozen condition for various intervals. The activity was tested on a hexosediphosphate-glucose substrate in a bicarbonate buffer. The results are shown in table 8.

TABLE 8. *Effect of freezing on activity of juice of Aerobacter indologenes*

| Age of juice in days                 | 1   | 3   | 4   | 5   | 7  | 11 |
|--------------------------------------|-----|-----|-----|-----|----|----|
| Activity in mm <sup>3</sup> gas/hour | 129 | 125 | 119 | 127 | 69 | 71 |
| Endogenous                           | 9   | 9   | 4   | 8   | 11 | 0  |

Test conditions same as in table 1.

It is evident that the juice may be stored frozen for at least 11 days with a loss of activity of less than 50 per cent.

The juice is quite sensitive to heat. If maintained at 40° C. for 3 minutes, its activity is reduced by 35 per cent; 15 minutes at 50° C. (coagulation) causes a loss of 67 per cent. The effects of various treatments are tabulated in table 9.

The work described has been done with juices prepared from *Aerobacter indologenes*. The method as applied to this organism has been used successfully with only slight modification to obtain active juices from *Escherichia coli*, *Aerobacter aerogenes*, *Citrobacter freundii* and *Clostridium butylicum*. The juice from *Cl. butylicum* was only slightly active,

TABLE 9. *Effect of physical and chemical treatment of juice*

| Treatment                             | Effect on activity*      |
|---------------------------------------|--------------------------|
| 1. Filtration                         |                          |
| a. Seitz                              | Complete inactivation    |
| b. Jena glass (3 auf 5)               | " "                      |
| c. Chamberland<br>(numbers 3 and 7)   | " "                      |
| 2. Freezing (11 days)                 | 45 per cent reduction    |
| 3. Heating                            |                          |
| a. 40° C.—3 minutes                   | 35 per cent reduction    |
| b. 50° C.—15 minutes<br>(coagulation) | 67 per cent reduction    |
| 4. Toluene, 5 per cent                | 80-90 per cent reduction |

\* Test conditions same as in table 1.

liberating about 30 mm<sup>3</sup> of gas per hour in the presence of boiled yeast juice. However, with some modifications a more active juice from *Cl. butylicum* could undoubtedly be obtained, especially if the grinding time were to be investigated more fully. All efforts to obtain a juice acting on hexosediphosphate from members of the genus *Propionibacterium* were



unsuccessful. These bacteria are very susceptible to the effects of grinding for after 5 minutes the activity of the cell-glass mixture is almost completely destroyed. No attempt has been made to obtain juices from organisms other than those described, but it is believed that with suitable modification, active extracts may be obtained from many species of bacteria by application of the technique described. The approximate activities of various juices are shown in table 10.

TABLE 10. *Activities of Several Bacterial Juices*

| Juice source                      | <i>Escherichia coli</i> | <i>Aerobacter aerogenes</i> | <i>Citrobacter freundii</i> | <i>Clostridium butylicum</i> * |
|-----------------------------------|-------------------------|-----------------------------|-----------------------------|--------------------------------|
| Activity mm <sup>3</sup> gas/hour | 150-250                 | 90-200                      | 100                         | 25-35                          |
| Endogenous activity               | 3-5                     | 3-13                        | 19                          | 2-3                            |

Experimental conditions same as in table 1.

\* Contains 0.1 ml. boiled yeast juice in addition.

#### SUMMARY

A technique for the preparation of active cell-free glucolytic enzymes based on the use of powdered glass in the proper proportions for the disruption of the bacterial cell is described. Juices active on mixtures of hexosediphosphate and glucose have been prepared from *Aerobacter indologenes*, *Aerobacter aerogenes*, *Escherichia coli*, *Citrobacter freundii* and *Clostridium butylicum*. Various dehydrogenases have been detected in juices of *Aerobacter indologenes*.

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# DESCRIPTION OF A DEXTRO-LACTIC ACID FORMING ORGANISM OF THE GENUS *BACILLUS*<sup>1</sup>

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Received November 23, 1939

Werkman and Andersen (1938) reported a new fermentation process for the conversion of glucose into d-lactic acid in which a facultative aerobic, spore forming organism was used. A detailed study of this organism has shown it to be a new species of the genus *Bacillus*. Owing to the large quantity of dextro-lactic acid formed by this organism in fermentation processes the name *Bacillus dextrolacticus* is proposed.

## ISOLATION AND STUDY

The organism was isolated from a flask of medium composed of malt sprouts, glucose and calcium carbonate which had been heated for 25 minutes at 10 pounds steam pressure. The flask was serving as an uninoculated control in an experiment determining the effect of various temperatures on the rate of fermentation. The flask was taken directly from the autoclave and placed in an incubator at 47° C. It was observed on the second day that a vigorous fermentation was in progress. A transfer was made to a tube of sterile medium for subsequent study and the fermentation was allowed to continue. Analysis of the fermented liquor showed that the sugar fermented had been converted to dextro-lactic acid. Microscopical examination of the culture which had been taken, indicated that a single morphological type was present. The culture was plated and replated on glucose agar. Only colonies of a single type appeared. Well isolated colonies were picked into malt sprouts medium. A study of these cultures showed them to be identical with the original culture, indicating that the latter was a pure culture. The following study was made on one of the cultures obtained from a well isolated colony. The purity of the culture has been maintained throughout this work.

## MORPHOLOGY AND STAINING REACTIONS

The size, shape and staining characteristics of this organism vary considerably depending upon the medium, the temperature, the pH, oxygen tension and other factors. When the organism is grown in a malt sprouts-glucose-CaCO<sub>3</sub> medium at 47° C. the cells are somewhat larger and less uniform in size than when grown on a nutrient agar slant. On malt agar the rods rapidly become Gram negative. The last few organisms remaining Gram positive grow into long cells, many reaching 50 microns or

<sup>1</sup> Journal Paper No. J-704 of the Iowa Agricultural Experiment Station, Ames, Iowa.  
Project No. 421.

longer. Some cells have been observed as long as 150 microns (Pl. I, fig. 1). If  $\text{CaCO}_3$  is added to the malt agar the cells remain Gram positive and uniform in size. In synthetic media which have thus far been developed for this organism, the cells are smaller and do not retain the stain by Gram's method as well as on nutrient agar. Very irregularly shaped rods have been found in aerated malt sprouts-glucose medium.

Grown on a nutrient agar slant aerobically at 47° C. for 24 hours, the rods are from 0.6 to 0.9 microns wide and 3 to 8 microns in length. Longer cells occur rather frequently. The cells occur singly, in pairs and short chains. The organism is motile by means of peritrichous flagella. One, two or three flagella are frequently found but as many as seven have been observed on a single cell. Leifson's (1938) method was used for staining the flagella (fig. 2). Sporulation occurs best in nutrient agar slant cultures. The spores are ovoid, sub-terminal to terminal and there is some swelling as sporulation progresses. Dorner's method has been found satisfactory for staining the spores (fig. 3). The ends of the rods are rounded. Gram's stain is retained (fig. 4) and under some conditions the cells stain unevenly. This is particularly noted at the time the cells are becoming Gram negative.

#### CULTURAL CHARACTERISTICS

Grown aerobically on a nutrient agar slant the growth is moderate, filiform, opaque, slightly raised, glistening, smooth, butyrous; under transmitted light the internal structure appears to be finely granular, and the appearance of the medium is unchanged. If glucose is added to the agar, the growth is luxuriant. Anaerobically there is no growth on nutrient agar but moderate growth on glucose nutrient agar.

In an agar stab, growth occurs only in the upper part and is best at the surface. If glucose is present, growth extends to the bottom of the stab.

Colonies on a nutrient agar plate are circular, entire, convex, granular and 1 to 3 mm. in diameter. Subsurface colonies are small.

Incubated at 42° C. a gelatin tube slowly becomes turbid with a scanty surface growth. If glucose is added to the medium, growth is much better. Gelatin is not liquefied.

Nutrient broth inoculated and incubated at 47° C. quickly becomes turbid with a moderate amount of sediment. Citric acid is not attacked.

#### PHYSIOLOGICAL CHARACTERISTICS

The organism grows between 20° C. and 62° C.; the optimal temperature range is from 45° C. to 53° C.

The organism is a facultative aerobe. To obtain the most rapid fermentation of large quantities of sugar, aeration has been found necessary in certain kinds of media. When soybean meal is used to promote growth, aeration is necessary for optimal rate of fermentation.

Litmus milk is rapidly reduced and coagulated. Inoculated from malt

sprouts-glucose  $\text{CaCO}_3$  medium, litmus milk is coagulated and reduced in 9 to 12 hours. As the culture ages a red band forms at the top. No gas is formed. If  $\text{CaCO}_3$  is added the litmus remains reduced and the curd is partially digested.

Nitrates are reduced to nitrites. Catalase is formed. Hydrogen sulphide is not produced in lead acetate agar or iron citrate agar. Indole is not formed in tryptophane broth. The Voges-Proskauer reaction is positive.

Dissimilation of carbohydrates was determined by adding the carbohydrates, sterilized at 10 pounds steam pressure for 10 minutes, to yeast extract buffered medium, inoculating with a water suspension of cells, incubating at 42° C. for 48 hours and 72 hours and titrating the acid produced with 0.1 N NaOH. Acid in large amounts is readily formed from glucose, levulose, mannose, galactose, rhamnose, arabinose, trehalose, cellobiose, melibiose, maltose, lactose, sorbitol, methylglucoside and salicin. Acid in smaller amounts or at a much slower rate is formed from raffinose, glycerol, sucrose, xylose, amygdalin, dextrin and soluble starch. No acid is formed from adonitol, mannitol, dulcitol, inulin, pectin and starch. Gas formation from any of the above carbohydrates could not be detected by use of Durham tubes, or from glucose in Smith tubes.

The principal and substantially only product of glucose dissimilation is dextro-lactic acid. In a medium consisting of 1 per cent soybean meal, 8 per cent glucose, and mineral salts, the d-lactic acid formed accounted for 94 per cent of the sugar. The optical activity of the acid has been determined on acid formed in various media under varying conditions. The acid has constantly been of the dextro type. Small amounts of 2,3-butylene glycol and traces of acetylmethylcarbinol, diacetyl, acetic acid and ethyl alcohol were also produced. The lowest pH reached in glucose broth was 4.06.

#### NUTRITIVE REQUIREMENTS

Observations on the nutritive requirements of this organism have shown that neither amino acids nor proteins are essential, provided ammonium salts and certain growth factors are present. The effects of riboflavin, thiamin, ether soluble factor of acidified yeast extract and amino acids when added to a basal medium have been determined. The results are shown in table 1. The basal medium consisted of glucose, mineral salts and sodium acetate as a buffer. There was no growth on the basal medium alone or with thiamin and flavin. When the yeast factor alone was added to the basal medium, growth was poor and successive transfers were not possible; however, if thiamin or riboflavin was also added, good growth was obtained on successive transfers. For the purpose of promoting growth, thiamin and riboflavin replace each other in the medium equally well, one or the other is necessary but not both. The yeast factor can be replaced by hydrolyzed casein or three amino acids: glutamic acid, arginine or cystine. Threonine is also beneficial and may be substituted for arginine. Further work is under investigation.



TABLE 1. *The effect of lactoflavin, thiamin and the acidic ether soluble fraction of yeast extract on acid production*

| Tube no. | Composition of medium  | cc. 0.1 N acid per 100 cc. |     |     |     |     |     |     |
|----------|--|----------------------------|-----|-----|-----|-----|-----|-----|
|          |  | Transfers                  |     |     |     |     |     |     |
|          |  | 1                          | 2   | 3   | 4   | 5   | 6   | 7   |
| 1        | Basal <sup>1</sup>   | 0.3                        |     |     |     |     |     |     |
| 2        | Basal <sup>1</sup> plus Et <sub>2</sub> OY <sup>2</sup>                                      | 5.1                        | 5.0 | 3.5 | 2.2 | 0.5 |     |     |
| 3        | Basal <sup>1</sup> plus Et <sub>2</sub> OY <sup>2</sup> plus thiamin                         | 5.5                        | 4.8 | 5.1 | 5.5 | 4.8 | 5.7 | 5.5 |
| 4        | Basal <sup>1</sup> plus Et <sub>2</sub> OY <sup>2</sup> plus flavin                          | 5.0                        | 5.1 | 4.8 | 5.5 | 4.4 | 5.5 | 5.7 |
| 5        | Basal <sup>1</sup> plus Et <sub>2</sub> OY <sup>2</sup> plus thiamin plus flavin             | 5.3                        | 5.2 | 5.5 | 5.0 | 5.1 | 5.8 | 5.3 |
| 6        | Basal <sup>1</sup> plus thiamin plus flavin  | 0.4                        |     |     |     |     |     |     |
| 7        | Basal <sup>1</sup> plus thiamin plus glutamic acid plus arginine plus threonine plus cystine | 5.6                        | 5.8 | 4.7 | 3.6 | 4.0 | 4.0 |     |
| 8        | Basal <sup>1</sup> plus thiamin plus hydrolyzed casein                                       | 5.0                        | 5.1 | 4.8 | 5.1 | 4.7 | 4.8 |     |

<sup>1</sup> Basal medium: 1 per cent glucose, mineral salts, 0.3 per cent (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 0.6 per cent NaOAC.<sup>2</sup> Ether soluble fraction of acidified yeast extract.

## DIFFERENTIATION FROM PREVIOUSLY DESCRIBED ORGANISMS

Many of the characteristics of this organism, particularly the sugar fermentations which differentiate it from other organisms have been re-checked after a year. In all cases the properties of the organism remained constant.

Horowitz-Wlassowa and Nowotelnov (1932) reported a spore forming organism which produces lactic acid. The organism described by these investigators differs from the one described here in motility, fermentation reactions, nutritive requirements and in other ways.

Of the organisms described in the literature, *B. dextralacticus* sp. nov. most closely resembled *B. coagulans* described by Hammer in 1915 and by Sarles and Hammer in 1932. Three strains of *B. coagulans* were obtained from Dr. Hammer for comparative studies and all four cultures were subjected to exactly the same growth conditions. Several distinct differences were found. The three strains of *B. coagulans* were all negative with respect to nitrate reduction and arabinose and sorbitol fermentations while *B. dextralacticus* was positive. The Voges-Proskauer test for acetylmethylcarbinol was strongly positive for *B. dextralacticus*, but very weak for *B. coagulans*. A marked difference in the rate of glucose utilization was found in fermentation studies. The rate for *B. coagulans* was approximately one-half that of *B. dextralacticus*.

## SUMMARY

A spore-forming rod shaped organism producing large quantities of d-lactic acid has been isolated and studied. The organism belongs to the genus *Bacillus* and appears to be a previously undescribed species for which the name *Bacillus dextralacticus* is proposed.

A complete description of the organism is given. Its growth conditions and nutritive requirements are indicated.

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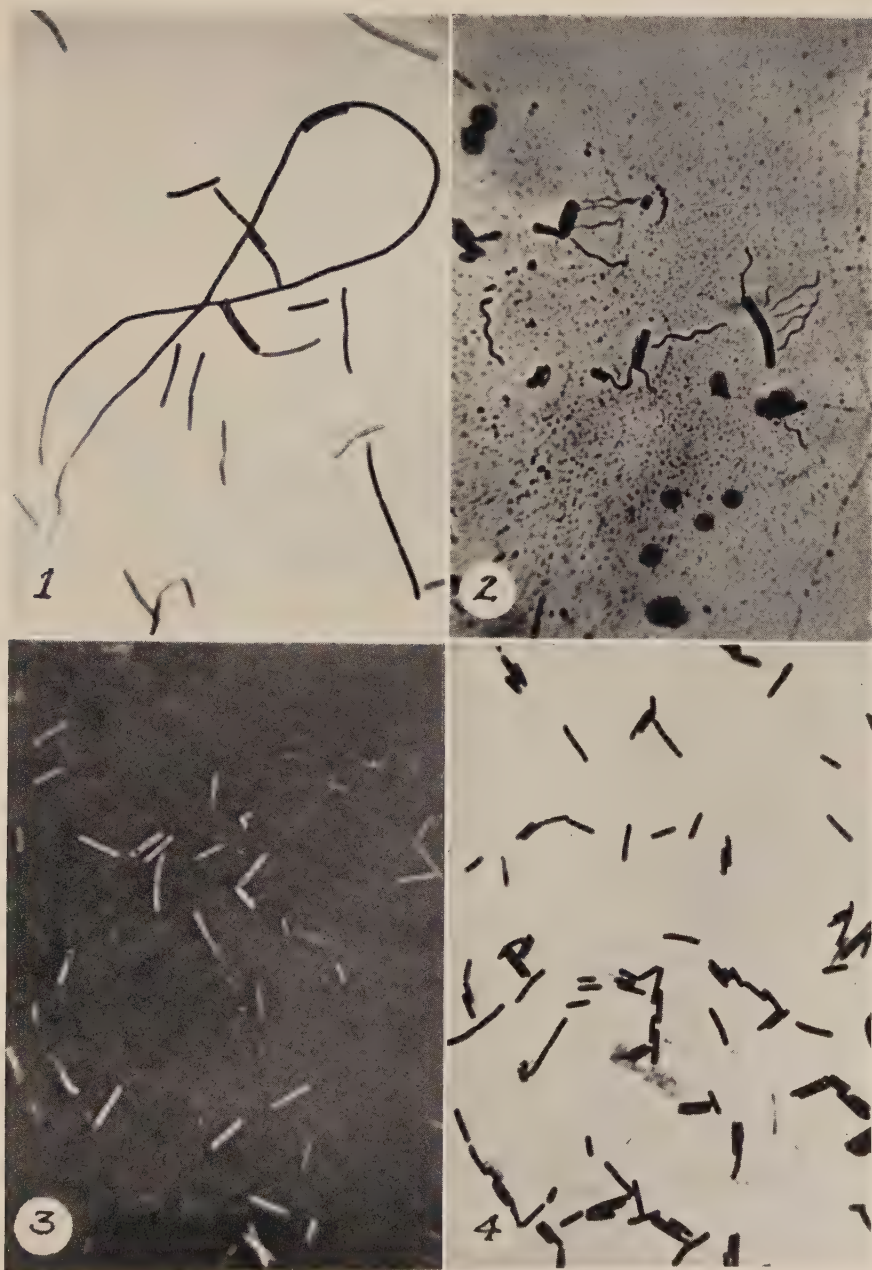
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## PLATE I

Photomicrographs of *B. dextralacticus*

- Fig. 1. Malt agar; 48 hours at 47° C.; Gram stain.  
Fig. 2. Flagella stained by Leifson's method.  
Fig. 3. Spores stained by Dorner's method.  
Fig. 4. Nutrient agar; 24 hours, 47° C.; Gram stain.

PLATE I







# EFFECT OF LOW TEMPERATURE ON THE INTENSITY OF FLUORESCENCE

J. E. DINGER AND WILLIAM KUNERTH

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Received November 24, 1939

During the last few years luminescent phosphors have become an important factor in artificial illumination. It is therefore desirable to study the properties of these phosphors under various conditions. A study has here been made of the effect of low temperatures upon the intensity of fluorescence; and upon the width of the fluorescent band.

## MATERIALS

The fluorescent materials used were commercial phosphors obtained from the Nela Park Laboratories of the General Electric Company. The phosphors were calcium tungstate ( $\text{CaWO}_4$ ), zinc silicate ( $\text{ZnSiO}_3$ ), and cadmium borate ( $\text{CdB}_2\text{O}_5$ ). As shown by Thayer and Barnes (1), these phosphors fluoresce with maximum intensities at the following wavelengths:

|                                |        |
|--------------------------------|--------|
| $\text{CaWO}_4$ .....          | 4400 A |
| $\text{ZnSiO}_3$ .....         | 5250 A |
| $\text{CdB}_2\text{O}_5$ ..... | 6150 A |

These phosphors are most efficient when the exciting radiation has a wavelength between 2200 A and 3000 A.

## APPARATUS

The ultra-violet radiation was obtained from a quartz mercury vapor arc, the 2537 A resonance line being used. A Gaertner constant deviation quartz monochromator with a slit width of two millimeters was used to select an intense beam of ultra-violet radiation. A Gaertner wavelength spectrometer (Pellin-Broca type prism) which had the telescope replaced by a spectroscopic camera attachment was used for photographing the fluorescent spectrum. An arrangement of the apparatus is shown in figure 1.

The fluorescent material was placed in a shallow inset in the top of a brass cone suspended below the slit of the monochromator and the slit of the spectrometer collimator. A bakelite insulating ring separated the quartz plate from the brass cone. A groove in the bakelite ring under the quartz plate and adjacent to the fluorescent material contained calcium chloride which removed the moisture immediately above the phosphor and thus prevented the formation of frost on the phosphor and the under side of the quartz plate. A Dewar flask containing liquid air was placed on a stand of adjustable height. By means of this stand the cone could be

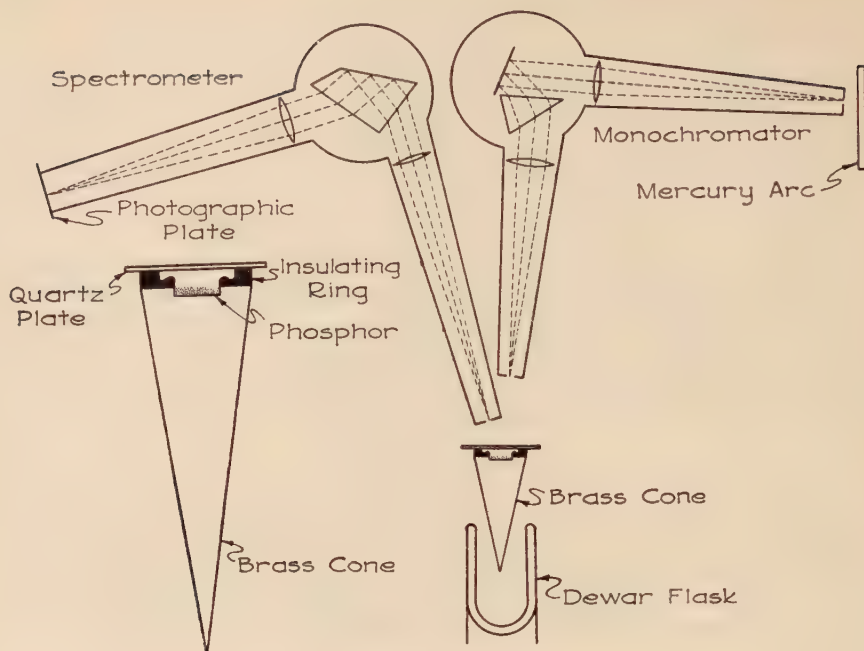


Fig. 1. Arrangement of apparatus.

immersed to various depths in the liquid air and in this way the temperature of the phosphor could be brought to any desired value between room temperature and the temperature of the liquid air. The formation of frost on the top of the quartz plate was prevented by passing a blast of dried air over the top of the plate.

A calibrated copper-constantan thermocouple was used for measuring the temperature of the phosphor.

#### METHOD

The temperature in the mercury arc housing was permitted to come to equilibrium and the current was maintained at a constant value in order to insure a constant output of ultra-violet radiation.

A series of either eight or sixteen exposures of the fluorescent spectrum of a phosphor was made on a photographic plate, the temperature of the phosphor being changed between exposures. A series of exposures was made as the temperature was lowered by steps (25 or 13 degrees centigrade, depending on the number of exposures on plate) from room temperature down to liquid air temperatures and another series as the temperature was permitted to rise to room temperature. Five minute exposures were used, it being possible to maintain the temperature of the phosphor within  $3^{\circ}\text{C.}$  of some desired value throughout this interval.

Microphotometer recordings were made on the density resulting from each exposure of the  $\text{ZnSiO}_3$  and  $\text{CdB}_2\text{O}_5$ . The height of the recording

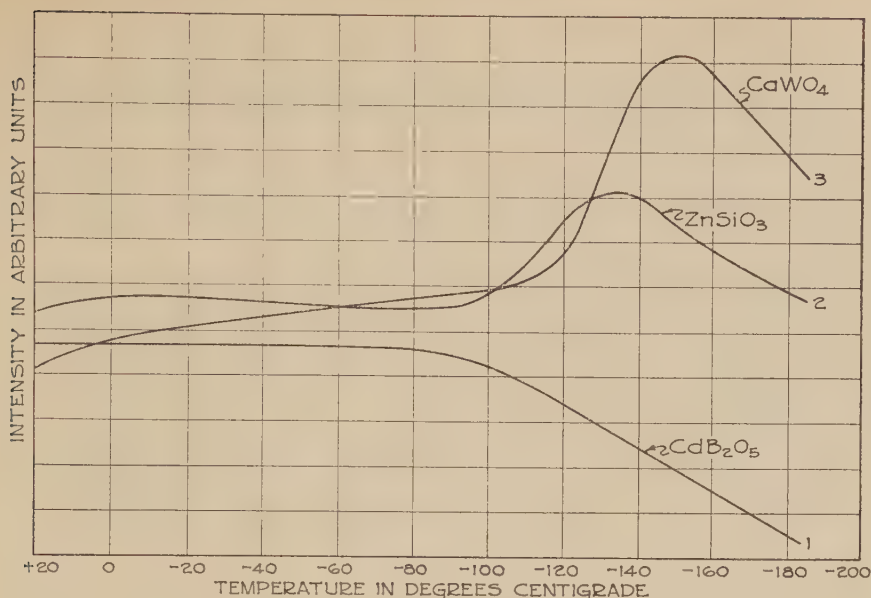


Fig. 2. Effect of temperature on intensity of fluorescence.

crest was taken as being proportional to the intensity of fluorescence. Because of the greater width of the  $\text{CaWO}_4$  band, microphotometer recordings were not made but instead maximum deflections of a galvanometer connected directly to the photoelectric cell of the microphotometer were taken as proportional to the intensity of fluorescence.

#### RESULTS

Curve 1, figure 2, indicates the variation of intensity of fluorescence with temperature as obtained from a number of measurements with  $\text{CdB}_2\text{O}_5$ . The intensity varies very little until a temperature of  $-100^\circ\text{C}$ .

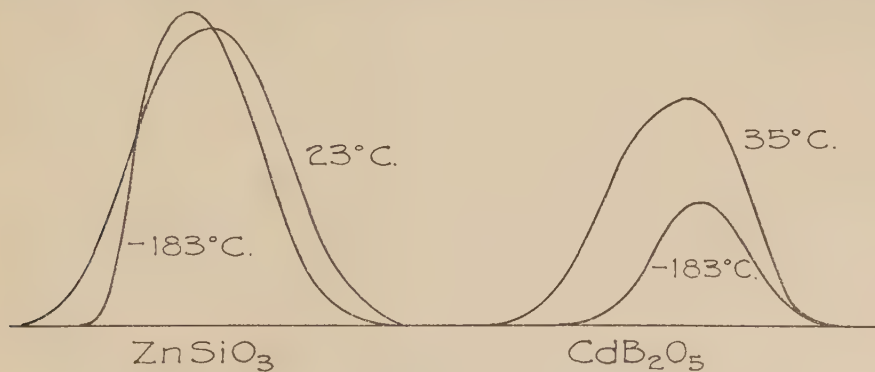


Fig. 3. Effect of temperature of band width.



is reached and a steady decline is noted as liquid air temperature is approached.

Curve 2, figure 2, shows the relation between intensity of fluorescence and temperature of the phosphor  $\text{ZnSiO}_3$ . The intensity reaches a maximum at about  $-140^\circ \text{C}$ .

$\text{CaWO}_4$  shows the most marked dependence of intensity of fluorescence upon temperature as indicated by curve 3, figure 2. There is a very marked increase in intensity from room temperature down to  $-150^\circ \text{C}$ . below which the intensity again decreases.

All three phosphors show a narrowing and sharpening of the band as the temperature is lowered. Figure 3 is a copy of two microphotometer recordings of each of the phosphors  $\text{ZnSiO}_3$  and  $\text{CdB}_2\text{O}_5$ . A spectrometer slit width of 0.15 mm. and an exposure time of 15 minutes was used in each case. The temperature of each phosphor was at the indicated temperature during the exposure which gave rise to the respective microphotometer recordings shown in figure 3. This narrowing of the band is to be expected and is in accordance with the theory as discussed by Milner (2).

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# A SUPPLEMENT TO THE CATALOGUE OF IOWA PLANTS IN THE IOWA STATE COLLEGE HERBARIUM<sup>1</sup>

ADA HAYDEN

*From the Botany and Plant Pathology Section, Iowa Agricultural Experiment Station*

Received December 1, 1939

The plants reported in this paper supplement the annotated list prepared by R. I. Cratty and published in 1933. The work of cataloging the flora of Iowa was begun by C. E. Bessey in 1871 based upon herbarium specimens and followed by the catalogue of Arthur in 1876. Other lists exist, which appear to be compilations. The plants here discussed are deposited in the herbarium at Iowa State College, though duplicates of most of them have been distributed elsewhere. Assistance was received in determination or verification of the following groups through the courtesy of Mrs. Agnes Chase for Gramineae, Dr. F. T. Hermann for *Carex*, Dr. H. K. Svenson for *Eleocharis*, Dr. Lyman B. Smith for *Comandra*, Dr. Robert Clausen for *Najas*, Mr. E. J. Palmer for *Crataegus*, Dr. Louis C. Wheeler for *Euphorbia*, Dr. E. D. Ball for *Salix*, Dr. E. W. Erlanson for *Rosa* in part, Dr. F. W. Pennell for *Penstemon*, and Dr. K. M. Wiegand for the *Aster paniculatus* group.

## ANGIOSPERMAE

### TYPHACEAE (Cat-tail Family)

*Typha angustifolia* L.

Narrow-leaved Cat-tail

In 6 ins. to 2 ft. of water, Barringer's Slough at the west end of Lost Island Lake, Clay County, Freeman Township., Sec. 1, July 10, 1934, Hayden 778; gravelly beach on west side of Lost Island Lake, Palo Alto Co., Highland Twp., Sec. 6, July 20, 1934, Hayden 772; abundant around the edges of New Lake, south of *Salix* about 10 miles south of Sioux City, Woodbury Co., Liberty Twp., Sec. 32, July 25, 1938, Hayden 11,229; swamp near Spirit Lake, Dickinson Co., Aug. 25, 1938, W. A. Anderson, M. L. Grant and A. Hayden 11,230; marshy south side of Rush Lake, Osceola Co., Fairview Twp., Sec. 39, H. S. Conard.

*Typha angustifolia* L. var. *elongata* (Dudley) Wiegand

Long-spiked, Narrow-leaved Cat-tail

A more robust form than the typical form. Seems more abundant than *T. angustifolia*. Both *T. angustifolia* and its form are more abundant locally in the shallow alkaline lakes of Clay and Palo Alto Counties than *T. latifolia* L. Intermediate types between *T. angustifolia* and *T. latifolia* also occur.

<sup>1</sup> Journal Paper No. J-662 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 366. In co-operation with the Bureau of Biological Survey (U. S. Dept. of Interior), the American Wild Life Institute and the Iowa State Conservation Commission.

## NAJADACEAE (Pondweed Family)

*Najas guadalupensis* (Spreng.) Morong

Guadalupe Naiad

See Rh. 25:107. 1923.

*Caulinia guadalupensis* Spreng.

Abundant along the margin of Mud Lake on the south side. Clay Co., Lake Twp., Sec. 36, July 7, 1934, Hayden 825; in shallow water of Round Lake, Clay Co., Freeman Twp., Sec. 3, Sept. 2, 1936, Hayden 827; in shallow water of the southwest bay of Trumbull Lake associated with *Potamogeton foliosus*, Clay Co., Lake Twp., Sec. 35, Aug. 13, 1837, Hayden 10,133.

## GRAMINEAE (Grass Family)

*Aristida dichotoma* Michx.

Forked Triple-awn Grass

Rocky, semi-open, wooded hillsides above the railroad tracks at Cliff-land on the east side of the Des Moines River. Wapello Co., Keokuk Twp., Sec. 11, Oct. 1, 1938, Hayden 10,963. This collection lies toward the northwest margin of the present range. It is not reported for Iowa in Hitchcock's Manual.

*Muhlenbergia asperifolia* (Nees and Meyen) Parodi

Scratch Grass

See Hitchcock Man. Grasses U.S. Misc. Pub. U.S.D.A. 200:366. 1935.

*Sporobolus asperifolius* (Nees and Meyen) Thurber

Edge of Mud Lake on the lake terrace in black clay loam. Dewey's Pasture, Clay Co., Lake Twp., Sec. 25, Aug. 8, 1935, Hayden 63. An earlier collection of this species made by L. H. Pammel (I.S.C. 111,526) in Story Co. 2 miles east of Nevada, Iowa, was reported by Cratty in the I.S.C. Catalogue of 1933 as *Sporobolus asperifolius*. It appeared to be rare and local. It has since been observed in northern Iowa to be regularly recurrent in zones around marshes and lakes in alkaline soil.

*Muhlenbergia brachyphylla* Bush

Short-leaved Muhlenbergia

Floodplains of the Little Sioux River. In shade or in open spaces along the river bank in Wabash loam. Clay Co., Peterson Twp., Sec. 33, Sept. 19, 1936, Hayden 614. This species is reported for Ind., Ill., Miss., Nebr., Kan., Okla., and Texas.

*Muhlenbergia foliosa* (Roem. and Schult.) Trin.

Leafy Muhlenbergia

Two miles east of Ruthven on the east bank of Round Lake in sandy loam. Common. Clay Co., Lake Twp., Sec. 34, Aug. 22, 1934, Hayden 37; on a shaded, rocky bank at the margin of a small stream on the south side of Lost Island Lake below Loesser's Point (Babcock Farm), Palo Alto Co., Highland Twp., Sec. 6, Sept. 21, 1936, Hayden 616; in low ground along the Des Moines River about 2 miles north of Eldon, Wapello County, Washington Twp., Oct. 3, 1938, Hayden 10,973.

*Heleocholea schoenoides* (L.) Host. Cat's-tail Grass

Growing in hard soil in front of a wholesale house north of the Rock Island railway tracks between 6th and 7th St. in Des Moines, Oct. 5, 1937, Hayden 4073. This grass is introduced from Europe and is reported in Hitchcock's Manual in Mass., Del., Mich., and Ill.

*Distichlis stricta* (Torr.) Rydb. Inland Saltgrass

Growing in gravel and cinders along the track of the C. & N.W. railroad track, 3 miles east of Boone, Boone Co., July 3, 1935, W. L. Tolstead, I.S.C. 151,018. This plant appears to be near the eastern edge of its present range.

*Agrostis palustris* Huds. Creeping Bentgrass  
*A. maritima* Lam.

Marshy ground around the outlet of Lost Island Lake known as Baringer's Slough, in wet, black soil, Clay Co., Lake Twp., Sec. 10, July 19, 1935, Hayden 265. The branches of the inflorescence were somewhat appressed. Other colonies were seen in Clay Co. along the marshy border of a stream known as Pickerel Run near Dickens.

*Echinochloa Crusgalli* (L.) Beauv. var. *mitis* (Pursh) Peterm. Awnless Barnyard Grass

Around ponds, lakes, moist depressions along roadsides and in pastures, growing with the awned variety. Common. Clay Co., Aug. 28, 1935, Hayden 121.

*Panicum auburne* Ashe The Auburn Panic Grass

Upland open woods south of Estherville, Emmet Co., June 29, 1936, Wolden, B. O. 1555. (First collected at Auburn, Ala.)

*Panicum clandestinum* L. Deer-tongue Panic Grass

One mile west of Floris. Large, luxuriant plants growing in a colony in a marshy area along Lick Creek on the Hill Culture Experimental Farm, Davis Co., Lick Creek Twp., Sec. 26, June 26, 1938, Hayden 10,951.

*Panicum Deamii* Hitchc. and Chase Deam's Panic Grass

Allamakee Co., Iowa Twp., Sec. 21. A pioneer plant growing on an open sand dune along the terrace of the Upper Iowa River Valley, 5-10 miles southwest of New Albin, Hayden 8014. Also W. L. Tolstead, I. S. C. 151,016 and 151,017. The distribution of this plant appears at present to be localized and appearing according to Hitchcock on sand dunes, in northern Indiana and Iowa.



## CYPERACEAE (Sedge Family)

*Eleocharis Engelmanni* Steud.

Engelmann's Spike Rush

Gitche Manito State Park, Lyon Co., Sept. 1934, I. E. Melhus, I. S. C. 142,409; growing in a muddy depression below a rock outcrop of Sioux quartzite in Gitche Manito State Park, Lyon Co., Sioux Twp., July 22, 1937, Hayden 7017; Clay Co., Lake Twp., Sec. 36, July 31, 1939, Hayden 9198.

*Eleocharis parvula* (R. & S.) Link. var. *anachaeta* (Torr.) Svenson

Dwarf Spike Rush

Mon. Stud. in *Eleocharis*. Rh. 36:386. 1934.*E. pygmaea* Torr. var. *anachaeta* Torr.; *E. leptos* Svenson*Scirpus nanus* var. *anachaetus* Britton

South sandy shore of Round Lake. This beach, revealed by the very low level of the lake caused by the recent protracted drouth is abundantly covered by a green carpet of *Eleocharis* including *E. acicularis*, *E. parvula* (R. & S.) Link. var. *anachaeta* (Torr.) Sven., *E. calva* Torr., and *Cyperus inflexus* Muhl. Abundant throughout August. Clay Co., Freeman Twp., Sec. 3, Aug. 30, 1936. Hayden 690.

*Eleocharis elliptica* Kunth.

Capitate Spike Rush

See Rh. 41:65. 1939.

*E. capitata* (L.) R. Br. var. *borealis* Svenson. Rh. 34:200. 1932.

In quaking bog, Emmet Co., Emmet Twp., Sec. 28, July 28, 1930. B. O. Wolden. I. S. C. 136,033. (Formerly listed as *E. tenuis*.) One of the southernmost stations west of the Mississippi.

Note on the *Palustris* group of *Eleocharis*: This group as it has been studied geographically has expanded into several concepts, comprehensively discussed in the monographic studies of Fernald and Brackett (1929) and Svenson (1939). The representatives mentioned in the I. S. C. catalogue were *E. palustris* (L.) R. & S. and *E. glaucescens* Willd., the filiform stemmed plant now called *E. calva* Torr. The sheets labelled *E. palustris* (L.) R. & S. fall under 3 species:

*Eleocharis macrostachya* Britton

Pale Spike Rush

This material is distributed in the following counties: Clay 6, Dickinson 1, Emmet 2, Fayette 1, Palo Alto 1, Harrison 1, Story 1, Winneshiek 1.

*Eleocharis pauciflora* (Lightf.) Link. var. *Fernaldi* Svenson

Fernald's Few-flowered Spike Rush

Rh. 36:380. 1934.

In bog, Emmet Co., Emmet Twp., June 24 and July 14, 1931. B. O. Wolden, I. S. C. 137,045, which was previously listed as *Scirpus pauciflorus*, Lightfoot.

*Eleocharis Smallii* Britton

## Small's Spike Rush

The distribution by counties is: Buena Vista 1, Chickasaw 1, Emmet 7, Palo Alto 1, Story 2, Webster 1, Wright 2.

Notes on Iowa *Eleocharis*: I. S. C. 88,102 and 137,644, formerly listed as *E. intermedia* Schultes, is now known as *E. reclinata*. It is still unproved that *E. reclinata* grows in Iowa.

*Scirpus pallidus* (Britton) Fernald. Rh. 8: 162. 1906.

## Pale Bulrush

*S. atrovirens* Muhl. var. *pallidus* Britton

Wet meadows, sloughs, marshes and springs. Clay Co. Hayden 160, 161, 162, 164, 1072; Palo Alto Co., Hayden 163; Story Co., Hayden 8054; Winneshiek Co. 401.

*Scleria verticillata* Muhl.

## Low Nut Rush

In a bog about 4 miles northwest of Estherville, Emmet Co., Emmet Twp., Sec. 28. About 4 miles north of Estherville. Wolden 1407.

*Carex praegracilis* W. Boott

## Clustered Field Sedge

See N. A. Flora 18: 35. 1931.

*C. camporum* Mack.

*C. marcida* Boott

Low terrace around Mud Lake, sandy to gravelly soil in Dewey's Pasture, Clay Co., Lake Twp., Sec. 25, July 20, 1935, and May 30, 1936. Sept. 18, 1936, Hayden 199, 237 and 700. Frequent in Clay and Palo Alto Counties in low prairie.

*Carex foena* Willd.

## Dry-spiked Sedge

See Rh. 40: 327. 1938.

*C. siccata* Dewey.

Forming a thick sod on a dry bank along the roadside from Wanata State Park to Peterson, at the north side of the C. & N. W. R. R. crossing. Clay Co., Peterson Twp., Sec. 33, June 1936, Hayden 652.

*Carex molesta* Mackenzie N. A. Flora 18: 150. 1931.

Dry rocky ground, Gitche Manito State Park, Lyon Co., Sioux Twp., June 21, 1937, Hayden 8085.

*Carex pennsylvanica* Lam. var. *digyna* Böck, Linnaea 41: 220. 1877.

*C. heliophila* Mackenzie, Torreyia 13: 15. 1913, also N. A. Flora 18: 196. 1935.

This variety seems recurrent through the range of *C. pennsylvanica* in Iowa but found usually in dry, open woodland or in dry, gravelly prairie or on roadside banks. Hayden 147, 172, 8061, 8062.

*Carex tonsa* (Fernald) Bickn.

Umbel-like Sedge

See N. A. Flora 18:205. 1935.

*C. umbellata* Schkuhr

*C. umbellata* Schkuhr var. *tonsa* Fern.

A pioneer plant in a sand dune blow-out on the river terrace of the Upper Iowa River, Allamakee Co., about 5 miles west of New Albin. Sept. 13, 1937, W. L. Tolstead, I. S. C. 151,020.

*Carex substricta* (Kukenth) Mackenzie

American Water Sedge

See N. A. Flora 18:398. 1935.

*C. aquatilis* Wahl.

*C. aquatilis* Wahl. var. *substricta* Kukenth.

Bog in Emmet Co., June 20, 1926, B. O. Wolden 1230; Clay Co., abundant in swamps, around bogs and prairie springs, in 6 in. to 1 ft. of water associated with *C. atherodes*, *C. laeviconica*, *C. lacustris*, *C. rostrata*, *Calamagrostis canadensis*, *Scirpus acutus* and *S. validus*, June and July, Hayden 234, 640, 653, 655, 656, and 8086; Palo Alto Co., similar locations; Hayden 642, 654, 771 and 8086.

#### SALICACEAE (Willow Family)

*Salix discolor* Muhl. var. *prinoides* (Pursh) Andersson

Chestnut-leaved Pussy Willow

*S. prinoides* Pursh

Margin of a swamp on the sand terrace of the Upper Iowa River Valley, Sept. 13, 1937, Ada Hayden 7048.

*Salix humilis* Marsh. var. *rigidiuscula* (Andersson) Robinson and Fernald

Narrow-leaved Prairie Willow

High, dry, morainal prairie slope along the bank of the M. & St. L. railway, Emmet Co., Estherville Twp., Sec. 23, Sept. 4, 1934, Hayden 1013; two or three miles south of Irvington in a large tract of level prairie near some kettle holes, Kossuth Co., Sherman Twp., Aug. 20, 1934, Hayden 1026; dry hilltop among native grasses 5 miles northeast of Ames, Apr. 20, 1934, May 2, 1934, Oct. 25, 1937. Hayden 990, 990a, 990b, and 990c.

The narrow-leaved variety of the species appears to be the prevailing form in Iowa.

*Salix interior* Rowlee var. *pedicellata* (Anderson) Ball

Pedicelled Sandbar Willow

Low ground between Round and Trumbull Lakes, Clay Co., Lake Twp., Sec. 35, Aug. 8, 1937, Hayden 6081; bordering a marsh 4 miles south of New Albin, Allamakee Co., French Creek Twp., Sec. 1, Sept. 13, 1937, Hayden 6086; along the south grassy margin of Johnson's Slough, one mile northeast of Lost Island Lake, Palo Alto Co., Lost Island Twp., Sec. 32, Aug. 10, 1938, Hayden 10,992.

This variety is commonly recurrent in the lake region of northern Iowa.

#### JUGLANDACEAE (Walnut Family)

*Carya Laneyi* Sarg. var. *chateaugayensis* Sarg.

Laney's Chateaugay Hybrid Hickory

Upland woods about 3 miles west of Ames, Story Co., Franklin Twp., Sec. 32. Oct. 18, 1936, and May 28, 1937. A. Hayden and C. C. Lounsberry 2000 and 2000a. This tree was located by Mr. Lounsberry while searching for native hickories suitable for propagation. This species is regarded as a hybrid of *C. cordiformis* and *C. ovata*. It has leaves like *C. cordiformis* and a sweet-kerneled fruit resembling that of *C. ovata*. It was first reported from Quebec and later from Ontario.

*Carya ovalis* (Wang.) Sargent var. *obcordata* Sarg.

Obcordate Small-fruited Hickory

See Trees and Shrubs 2:208. 1913.

*C. microcarpa* Nutt., in part.

*Hicoria microcarpa* (Nutt.) Britton

Seven miles northeast of Ames in a hickory grove (formerly of 200 trees) on top of a hill east of Soper's Mill Bridge and  $\frac{1}{4}$  mile west of Pleasant Grove Church. Infrequent but recurrent. Story Co., Milford Twp., Sec. 7. Oct. 18, 1936, and May 31, 1937. A. Hayden and C. C. Lounsberry 2015a.

*Carya ovalis* (Wang.) Sarg. var. *odorata* Sarg.

Fragrant Small-fruited Hickory

See Trees and Shrubs 2:208. 1913.

*C. microcarpa* Nutt., in part.

*Hicoria microcarpa* (Nutt.) Britton

A slender tree about 8 inches in diameter in the west side of the cemetery of Iowa State College in an oak-hickory, upland wood bordering Clear Creek. Story Co., Washington Twp., Sec. 4, Oct. 10, 1936, and May 27, 1937, A. Hayden and C. C. Lounsberry 1096 and 1096a. The tree was located by Mr. Lounsberry.

The distribution of *Carya ovalis* (Wang.) Sarg. is listed in Sargent's Trees of North America (1905) as Ames, Story Co., and southward to N. C. and Tenn. There is no specimen in the I. S. C. herbarium of an earlier date than the above. Malcolm McDonald (1937) lists one collected by E. W. Graves for the herbarium of Parsons College, Fairfield, Iowa.

#### BETULACEAE (Birch Family)

*Ostrya virginiana* K. Koch f. *glandulosa* (Spach) Macbr.

The Glandular Hop-hornbeam

See Field Mus. Publ. Bot. 4:192. 1929.



Woodland slopes along the Little Sioux River, Clay Co., Gillett's Grove Twp., Sec. 25. June 27, 1936, Hayden 1834. Other sheets in the I. S. C. herbarium recently determined by Wm. Brandau under the direction of G. J. Goodman show the following distributions for counties: Allamakee, 2; Black Hawk, 1; Clayton, 4; Floyd, 1; Grundy, 1; Hardin, 1; Kossuth, 1; Lee, 1; Sac, 1; Van Buren, 2; Winnebago, 1. Out of the 160 specimens in the herbarium about 10 per cent can be referred to the glandular variety.

#### SANTALACEAE (Sandalwood Family)

*Comandra Richardsiana* Fern.  
See Rh. 7: 48. 1905.

Richard's False Toadflax

Three miles west of Floris and 1 mile north on Hwy. 63. Open wooded slope about  $\frac{1}{4}$  mile from the highway, Davis Co., Lick Creek Twp., Sec. 17. May 12, 1939, Hayden 9177; semi-open wooded ridge along Pease Creek in the Ledges State Park, Boone Co., Worth Twp., Sec. 16. June 6, 1939, Hayden 9178. This species is most frequently found in Iowa on open woodland ridges.

#### POLYGONACEAE (Buckwheat Family)

*Polygonum opelousanum* Riddell  
See Rh. 28: 27. 1926.

Opelousa's Knotweed

*Persicaria opelousana* (Riddell) Small

Abundant locally, in an ox-bow pond near the Raccoon River one mile north of Panora, Guthrie Co., Cass Twp., Sec. 29, July 15, 1932, Hayden 3068. Growing in 2 ft. of water in a pond 1 mile northwest of Panora, Guthrie Co., Cass Twp., Sec. 31 Sept. 5, 1933, Hayden 969.

*Polygonum natans* A. Eaton  
See Rh. 27: 158. 1925.

*P. amphibium* of Am. Auth. not L.

*Persicaria amphibia* S. F. Gray

*Polygonum natans* A. Eaton f. *Hartwrightii* (Gray) Stanford

Abundant locally, in an ox-bow pond near the Raccoon River one mile north of Panora, Guthrie Co., Cass Twp., Sec. 29, July 15, 1932, Hayden 3068; growing in 2 ft. of water in a pond 1 mile northwest of Panora, Guthrie Co., Cass Twp., Sec. 31. Sept. 9, 1933, Hayden 969.

*Polygonum coccineum* Muhl. var. *rigidulum* (Sheldon) Stanford  
See Rh. 27: 165. 1925.

*P. rigidulum* (Sheldon) Stanford

*Polygonum coccineum* Muhl. f. *natans* (Wiegand) Stanford

*Polygonum coccineum* Muhl. f. *terrestre* (Willd.) Stanford

The Water Polygonums in the Catalogue of Iowa Plants were listed in nomenclature which related to the European species. While European

species resembling the above do occur in North America they appear to be confined to northeastern America and are not common.

A considerable collection of the above varieties and forms were accumulated by the writer since 1932 when studying the life history of the role of the group as weeds and as food of waterfowl at Huxley in Story Co., Panora in Guthrie Co., and the Ruthven area in Clay and Palo Alto Counties. There are also other sheets from a number of counties.

#### ILLECEBRACEAE (Knotwort Family)

*Anychia polygonoides* Raf. Forked Chickweed

Abundant on sandy cliffs above Cliffland on the east side of the Des Moines River, Wapello Co., Keokuk Twp., Sec. 11, Oct. 2, 1938, Hayden 11,360.

#### RANUNCULACEAE (Crowfoot Family)

*Cimicifuga racemosa* (L.) Nutt. Bugbane, Black Cohosh

Infrequent in low, shaded woods in Stone Park northwest of Sioux City along the Big Sioux River, Woodbury Co., Sioux City Twp., July 21, 1938, Mrs. C. T. Englehardt and A. Hayden 10,703.

#### LAURACEAE (Laurel Family)

*Sassafras albidum* (Nutt.) Nees var. *molle* (Raf.) Fern. Sassafras

See Rh. 38:178-179. 1936.

*S. officinale* Nees and Eberm.

*S. variifolium* (Salisb.) Ktze.

Occurring in a colony on a rocky wooded ridge above Highway 61 just before it crosses the Des Moines River southwest of Keokuk. Lee Co., Jackson Twp., Sec. 33. Oct. 27, 1939, Hayden 9091.

#### ROSACEAE (Rose Family)

*Crataegus pruinosa* (Wendl.) K. Koch Waxy Thorn

*C. conjuncta* Sarg.

*C. depressa* Ashe, not Presl, in part.

*C. patrum* Sarg.

Upland woods  $\frac{1}{2}$  mi. west of the east entrance to the Ledges State Park. A small tree. Boone Co., Worth Twp., Sec. 21. May 20, 1936, Hayden 11,058.

*Potentilla missurica* Hornem. Great Plains Cinquefoil

See Rh. 37:291. 1935.

*P. pennsylvanica* L.

*P. strigosa* Pallas

Not infrequent locally around outcrops of Sioux quartzite rock in the prairie of Gitche Manito State Park. Lyon Co., Sioux Twp., June 22,

1931, Hayden 10,459. This species occurs on the plains of Man.-Kans.-N.M.-B.C. and the station in Iowa lies in the eastern border of its range.

*Geum vernum* (Raf.) T. & G.

Early Water Avens

On wooded slopes of the Des Moines River, 16 miles west of Oska-loosa, Mahaska Co., Scott Twp., Sec. 19, April 20, 1938, Hayden 11,284; wooded slopes 2 miles southwest of Cliffland, Wapello Co., Keokuk Twp., Sec. 16, April 26, 1938, Hayden 11,285; wooded slopes on the Hill Culture Experimental Farm, Davis Co., Lick Creek Twp., Sec. 15, June 25, 1938, Hayden 11,286; Jefferson Co., at Fairfield, May, 1928, L. H. Pammel, I.S.C. 135,001. The last collection was discovered in the herbarium by Dr. G. J. Goodman in 1938, under a different determination.

*Rose setigera* Michx. var. *tomentosa* T. & G.

Woolly Prairie Rose

Collected in the vicinity of Audubon by E. M. Cole who contributed a plant which is now growing on the Campus of Iowa State College. Audubon Co., June 28, 1935, E. M. Cole and A. Hayden 9,181.

*Rosa setigera* Michx. var. *serena* Palmer and Steyermark

Thornless Prairie Rose

Growing in the woods near Columbus Junction on the Weber property. A plant presented by Geo. L. Weber is now growing on the campus of Iowa State College. Louisa Co., Concord City Twp., June 25, 1933, G. L. Weber and A. Hayden 9,180.

*Rosa rudiusscula* Greene

The Rod-stemmed Rose

See Bot. Gaz. 96: 251. 1934.

Open upland about  $\frac{1}{2}$  mile west of the entrance to Pike's Peak Park, Clayton Co., Mendon Twp., Sec. 27. June 18, 1932, Sept. 18, 1932, Hayden 11,570; upland about  $\frac{1}{4}$  mile west of the entrance to Pike's Peak Park, fruits sparsely, a very handsome rose with shining foliage, large buds and flowers, Clayton Co., Mendon Twp., Sec. 27. June 18, 1932; Sept. 18, 1932, Hayden 11,571; growing along the right-of-way of the C. & N. W. R.R., Story Co., Washington Twp., Sec. 4. Sept. 15, 1932, Hayden 11,572; about 6 miles southeast of Lansing on a sloping roadside near a stream at the edge of the woods, a tall drooping form, Allamakee Co., La Fayette Twp., Sept. 18, 1932, Hayden 11,569; at the west edge of Marshalltown, along a roadside bank on Hwy. 30, Marshall Co., Marietta Twp., Sec. 26; June 20 and Sept. 17, 1933, Hayden 11,621.

This species, which according to Erlanson is a hybrid of *R. arkansana* Porter and *R. carolina* L., is rather variable but frequently recurring in the zone where *R. arkansana* and *R. carolina* occur. Its distribution seems to be most abundant in eastern to central Iowa.

*Prunus mahaleb* L.

Mahaleb Cherry

Tree 8 in. in diameter in a ravine at the southwest corner of Keokuk,

near the Des Moines River Bridge. Lee Co., Jackson Twp., Sec. 36, July 1935, H. S. Conard I.S.C. 151,015.

*Prunus nigra* Ait.

Black Plum

Small trees 5-12 ft. in height growing along the rocky bank of Davis Creek which flows through a rocky valley. In the open between hedges of *Salix cordata* and *S. interior* bordering the stream and an oak-hickory forest which covers high hills, Boone Co., Worth Twp., Sec. 16, Apr. 1935, Hayden 9,182; also Marcus Maxon No. 1, May 10, 1936.

#### LEGUMINOSAE (Bean Family)

*Vicia americana* Muhl. var. *truncata* (Nutt.) Brewer

Truncate-leaved American Vetch.

Low prairie east of Mud Lake, Clay Co., Lake Twp., Sec. 25, May 15, 1934, Hayden 4,071; moist black soil, Hughes prairie, Palo Alto Co., Lost Island Twp., Sec. 20., May 5, 1936, Hayden 4,070.

*Lathyrus venosus* Muhl. var. *intonsus* Butters and St. John

Hairy-leaved Veiny Vetch

See Rh. 19:158. 1917.

About 3½ miles west of Ruthven and 1 mile north of Hwy.18 along a willow hedge. Clay Co., Freeman Twp., Sec. 16, June 27, 1938, Hayden 11,315.

#### EUPHORBACEAE (Spurge Family)

*Euphorbia dentata* Michx.

Dentate-leaved Spurge

Abundant locally along a roadside bank in sandy loam, also growing in fields beside *Liatris punctata*. Cherokee Co., Pilot Twp., Sec. 22. Sept. 8, 1937, Hayden 6,029.

*Euphorbia Geyeri* Engelm.

Geyer's Spurge

Abundant locally on an open sand dune in the Upper Iowa River Valley about 5 miles southwest of New Albin, Allamakee Co., Iowa Twp., Sec. 21. Tolstead, W. L. and Hayden, A. 6,036.

This plant associated with *Polygonella articulata* Meisn., *Carex tosa* (Fern.) Bickn., *Panicum Deamii* Hitchc. and Chase, were pioneering vigorously with temporary success in an open dune in this vicinity, which has a local reputation for a long period of activity.

#### ONAGRACEAE (Evening Primrose Family)

*Oenothera perennis* L.

Small Sundrops

See Bull. Torr. Bot. Club. 64:287-304. 1937.

*O. pumila* L.

In upland wet prairie land and one mile east of Ridgeway. Winne-



shiek Co., Lincoln Twp., Sec. 24, July 29, 1933, W. L. Tolstead I.S.C. 145,068.

#### VIOLACEAE (Violet Family)

*Viola eriocarpa* Schwein. var. *leiocarpa* Fernald and Wiegand  
Smooth Yellow Violet

See Rh. 23:275. 1922; also Bulletin Bot. Club 38:194. 1911.

*Viola scabriuscula* Schwein. in part.

When identifying some plants from Clay and Palo Alto Counties, it was noticed that the ovaries were smooth instead of hairy. The distribution of the variety with the smooth ovary by counties is: Chickasaw, 1; Cerro Gordo, 1; Clay, 5; Crawford, 2; Decatur, 1; Emmet, 8; Delaware, 2; Fayette, 2; Hamilton, 1; Hardin, 4; Marion, 1; Marshall, 1; Palo Alto, 2; Lyon, 1; Story, 10; Van Buren, 1; Wapello, 1; Webster, 1; Winnebago, 2; Wright, 1; Winneshiek, 3.

*Viola missouriensis* Greene Missouri Violet

Sandy moist alluvial soil along a willow-margined stream, color bluish-white, sepals narrowly white margined, Story Co., Franklin Twp., Sec. 16, May 12, 1935, Hayden 10,179; about 2 miles south of Algona in low, sandy wooded hills along the Des Moines River, Kossuth Co., Irvington Twp., May 15, 1937, Hayden 10,256; sandy soil along a stream under willows just south of Cliffland below the hills along the Des Moines River, Wapello Co., Keokuk Twp., April 26, 1938, Hayden 11,387. Newbro finds this species frequent in Iowa, though not as common as *V. papilionacea*.

*Viola viarum* Pollard Plains Violet

At the edge of upland oak-hickory woods east of Ogden, Boone Co., Yell Twp., Sec. 35, May 15, 1932, Hayden 11,397; infrequent in open woods southwest of Cliffland, Wapello Co., Keokuk Twp., Sec. 15, April 26, 1938, Hayden 11,366; also along roadside bank 3 miles northwest of Eldon, Hayden 9,187; Davis Co., Lick Creek Twp., Sec. 9, about 2 miles west and 2 miles north of Floris scattered in somewhat shaded bluegrass sod in the cemetery beside a log church (an early Baptist church in Iowa), May 12, 1939, Hayden 9,188. On lawn at Onawa, Monona Co., April 18, 1938, Mrs. Harve Taylor I.S.C. 147,170. Newbro (1936) reports this species as rather uncommon, occurring in the western part of the state on prairie and dry exposures.

#### HALORAGIDACEAE (Water Milfoil Family)

*Hippuris vulgaris* L. Mare's-tail

A single colony occurred in a marsh with *Scirpus validus* and *Sparganium eurycarpum*, Dewey's Pasture, 7 miles north of Ruthven, Clay Co., Lake Twp., Sec. 25., June 25, 1936, Hayden 779.

## SCROPHULARIACEAE (Figwort Family)

*Penstemon albidus* Nutt.

Pale Beard-tongue

Gravelly hills north of Oak Grove State Park along the Little Sioux River. Locally abundant. Sioux Co., Buncombe Twp., July 23, 1937, Hayden 9,033. This location is on the eastern border of the distribution of this great plains species according to the map of Pennell (1935).

*Penstemon gracilis* Nutt.

Slender Beard-tongue

Common on dry prairie knolls and slopes of Gitche Manito State Park and vicinity, Lyon Co., Sioux Twp., June 22, 1937, Hayden 9,032. This station is on the southeasterly margin of its range, though it reaches farther north and east than *P. albidus*.

## CAPRIFOLIACEAE (Honeysuckle Family)

*Sambucus canadensis* L. var. *submollis* Rehd.

Pubescent Elder

In Sargent Trees and Shrubs 2:188. 1911.

Low border of woods on the Hill Culture Experimental Farm one mile west of Floris. Davis Co., Lick Creek Twp., Sec. 26, June 26, 1938, Hayden 11,481.

## LOBELIACEAE (Lobelia Family)

*Lobelia siphilitica* L. var. *ludoviciana* A. DeCandolle

Smooth Great Lobelia

See Rh. 38:281. 1936.

*L. siphilitica* A. DC. in part.

Low ground Fayette, Fayette Co., July 30, 1934, Bruce Fink, I.S.C. 26,564. A duplicate of this sheet was cited by McVaugh (1936) as typical of the Mississippi Valley smooth variety. About 40 sheets in the I.S.C. herbarium appear referable to this variety.

*Lobelia spicata* Lamarck var. *hirtella* Gray

Hairy Spicate Lobelia

See Rh. 38:313. 1936.

*L. spicata* Lamarck in part.*L. hirtella* Greene.

Low prairies, Aromstrong, Emmet Co., Aug. 13, 1898. I.S.C. 22,574; Ames, June 25, 1897, C. R. Ball & R. Combs 500. Duplicates of these sheets were cited by McVaugh (1936). About 30 sheets from the eastern three-fourths of the state are represented by this variety.

## COMPOSITAE (Sunflower Family)

*Aster amethystinus* Nutt.

Amethyst Aster

About 5 miles north of Ruthven in Dewey's Pasture, in moist soil near a pond. Another cluster grew on the margin of a swale west of the

hill above Mud Lake. *Aster nova-angliae* and *A. ericoides* (*A. multiflorus*) were near and abundant; Clay Co., Lake Twp., Sec. 25, Sept. 14, and Sept. 20, 1936; Sept. 15, 1937, Hayden 11,133, 11,134 and 11,137; in moist hummocky soil on the west side of the west lagoon southwest of Virgin Lake, Palo Alto Co., Highland Twp., Sec. 25, Sept. 16, 1938; the large plants growing in Dewey's Pasture for several years disappeared in 1938. They grow at a fluctuating water level, which may have a bearing on their ability to persist in the same location.

*Aster paniculatus* Lam. var. *simplex* (Willd.) Burgess

A collections of about 20 specimens taken mostly in northern Iowa, were referred to this variety of *A. paniculatus*. Their determination was based on corolla and leaf measurements required by Wiegand's monograph (1935). This variety is predominant in the state.

*Aster lateriflorus* (L.) Britton var. *pendulus* (Ait.) Burgess

Pendant Lateral-flowered Aster

This aster is common to abundant on shaded woodland slopes and floodplains. The following may be cited: Davis Co., Lick Creek Twp., Sec. 26, one mile west of Floris on the Hill Culture Experimental Farm, Oct. 1, 1938, Hayden 11,146; Boone Co., Cass Twp., Sec. 33, Sept. 26, 1938, Hayden 11,148. It appears to be of more frequent occurrence than the typical variety.

*Ambrosia longistylis* Nutt.

Long-styled Ragweed

At the edge of a dry pasture at the entrance to the bridge over the west fork of the Des Moines River west of Osgood, Palo Alto Co., Walnut Twp., Sec. 34, Oct. 13, 1936, Hayden 10,709; 10 miles south of New Albin growing on a sandy river terrace, Allamakee Co., Sept. 15, 1937, W. L. Tolstead.

*Ratibida calumnaris* (Sims.) D. Don f. *pulcherrima* (DC.) W. M. Sharp, Ann. Mo. Bot. Gard. 22:70. 1935. Long-headed Cone-flower

*Lepachys columnaris* (Sims.) D. Don var. *pulcherrima* (DC) T. & G.

Sandy bank along highway 65 at the north edge of Des Moines, Polk Co., Saylor Twp., Sec. 21. June 26, 1937, Hayden 10,546.

*Verbesina enceloides* (Cav.) B. & H. var. *exauriculata* Robinson and Greenm. Golden Crownbeard

*Ximenesia exauriculata* (Rob. and Greenm.) Rydb.

A single plant on the margin of a field 2 miles north of Ruthven north of the Lake School, Palo Alto Co., Highland Twp., Sec. 6, Sept. 1, 1934, Hayden 10,550; a patch 2 miles north of Iowa State College Campus at the edge of a barnyard, Story Co., Franklin Twp., Sec. 28, Oct. 1, 1934, A. Hayden and W. L. Tolstead 8189; introduced in pastures, Emmet Co., Ells-

worth Twp., Sept. 25, 1934, B. O. Wolden, I.S.C. 151,019.

*Hieracium scabriusculum* Schwein.

Narrow-leaved Hawkweed

Wooded slopes along Brown Creek southeast of Estherville, Emmet Co., Estherville Twp., Aug. 10, 1934, Hayden 10,600; open woods along Pease Creek, Ledges State Park, Boone Co., Worth Twp., Sec. 16, Sept. 1935, Hayden 9190; Woodman's Hollow, in woods, Webster Co., Cooper Twp., Sec. 1, Aug. 15, 1936, Marcus Maxon 4.

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# THE EFFICACY OF ULTRA-VIOLET LIGHT SOURCES IN KILLING BACTERIA SUSPENDED IN AIR

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Received September 13, 1939

Wells and Fair<sup>1</sup> have shown that ultra-violet radiations are effective in destroying bacteria suspended in air. This paper reports the results of a quantitative investigation of the factors involved in this bactericidal action. The investigation was carried out in the Sanitary Engineering laboratories of Harvard University, under the direction of Professor G. M. Fair.

The first approach to a solution of the problem was made by the author and W. F. Wells by sampling infected air as it approached an ultra-violet light in a tunnel. This work led to the development, by the author, of apparatus on a laboratory scale. The final equipment used was a duct system in which the infected air could be sampled at several points as it approached the ultra-violet light source at a constant velocity.

## APPARATUS

A diagram of the apparatus used is shown in figure 1. It will be described by following the air flow through the system. Room air passes into a 12-inch-diameter duct. At the point of entrance a dilute culture of bacteria is sprayed into the air by an atomizer and another atomizer humidifies the air to the required value. The air then passes through twelve feet of duct and through a mixing tank provided with baffles. From this tank the air enters the irradiation duct which is a 12-inch-diameter duct 16-feet-long leading into the tank in which the ultra-violet light source is placed. The air passes from the light tank into a section of duct provided with straighteners and an orifice for measuring the flow. Beyond the orifice is a butterfly valve and then a blower for controlling and creating the air flow. From the blower the air is exhausted to the outdoors. Inserted in a tube leading from the entrance of the irradiation duct to the inlet of the blower are a wet bulb thermometer and a dry bulb thermometer for measuring the humidity of the air. At points 1, 2, 4, 8, and 16 feet from the ultra-violet light, perforated copper tubes are placed diametrically across the irradiation duct. The two ends of each tube are joined outside the duct and lead, through a small Venturi tube, to the air sampler, a Wells Air Centrifuge<sup>2</sup>.

The dosing atomizer takes its supply of culture from a constant level supply bottle which is in turn kept filled from a main supply bottle. The atomizer does not spray the culture directly into the air stream, but into a horizontally placed Erlenmeyer flask provided with side outlets. The coarse droplets impinge on the sides of the bottle and are drained away,

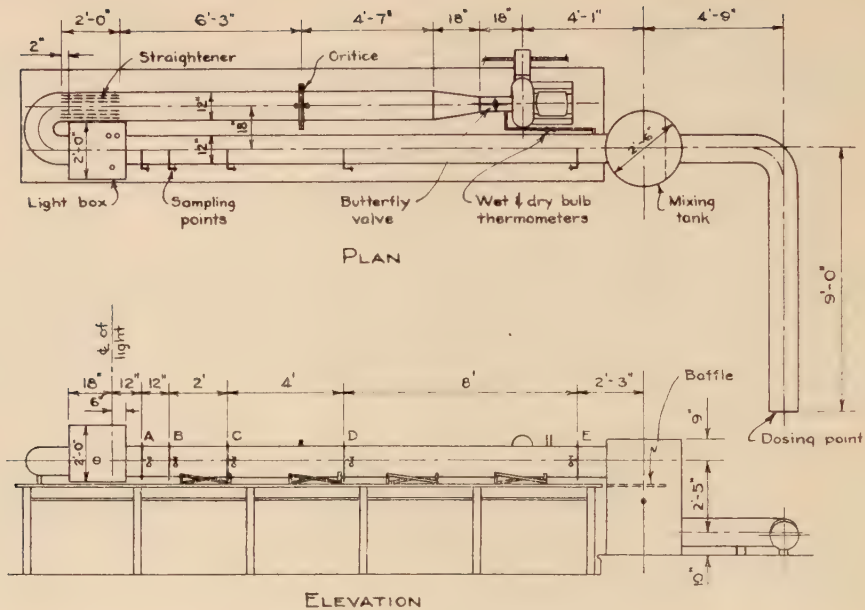


FIG. 1. Diagram of duct system.

while the finest droplets pass out the side outlet into the air stream where they evaporate, leaving minute infected particles floating in the air. The compressed air supply for the atomizer is kept at a constant pressure.

#### SOURCES OF ULTRA-VIOLET RADIATIONS

As work by Gates<sup>3</sup> and others on the irradiation of bacteria upon solid media had indicated that the effective range of radiations lies below a wave length of 302 mμ, light sources particularly effective in that region were investigated. Four light sources were used: (1) A high pressure quartz mercury arc known as the Uviarc, (2) a low pressure quartz mercury arc which was similar in design to a Cooper-Hewitt lighting unit, but only 14 inches long, (3) a Hanovia Alpine quartz mercury arc, and (4) a carbon arc, operated both with plain carbons and with therapeutic carbons.

#### BACTERIA INVESTIGATED

The greater part of the work was carried out using *Escherichia coli* as the experimental organism. Though not normally found in air, it was used because it is a non-pathogenic, easily cultivated, single celled organism whose cells do not attach themselves to each other. Its general characteristics are well known and it may be grown on a medium which inhibits the growth of other organisms which might interfere with the counts.

A few runs were made on each of three other species of bacteria. *Sarcina lutea* was used because, in contrast to *Escherichia coli*, it is a normal inhabitant of the air and it forms tenacious packets containing as many as 64 cells. *Staphylococcus aureus* was used because it is one of the organisms which should be removed from the air by any air disinfection method. It is a normal inhabitant of the air and is pathogenic, causing boils, abscesses and wound infections. It grows as single cells and in groups, usually in the form of plates. The third was *Bacillus subtilus* which differs from the other organisms used, in that it forms spores. It is a normal inhabitant of the air and is non-pathogenic.

#### EXPERIMENTAL PROCEDURE

Throughout the experiments the general procedure was as follows:

- a. The velocity of air flow in the irradiation duct was adjusted to a predetermined value.
- b. The ultra-violet light was started and permitted to warm up.
- c. The dilute culture of bacteria was placed in the supply bottle and the atomizer started.
- d. Six successive samples were taken from the 16, 1-, 2-, 4-, 8-, and 16-foot points in the order named.
- e. One variable was changed and the procedure repeated.
- f. The sample tubes were incubated at 37° C. for 24 hours and the developed colonies counted.

During each run the following data were recorded:

- a. The manometer readings from which the flow of air in the system was calculated.
- b. The voltage drop and current through the ultra-violet light source.
- c. The wet and dry bulb thermometer readings.
- d. The identifying mark on the sample tube.
- e. The duration, time of starting of the sample, and the rate of air flow in the sampler.

#### THEORETICAL ANALYSIS OF KILLING

Following the usual laws of disinfection, the killing effect of ultra-violet light upon bacteria in air may be expressed as follows:

$$dN = -kNdt \quad (1)$$

In this equation  $N$  is the number of bacteria present in a unit volume of air at a given instant,  $dN$  is the change in  $N$  in the time interval  $dt$ , and  $k$  is a constant depending upon the light intensity and the particular species

of bacteria being irradiated. This constant  $k$  may be replaced by  $\frac{k_0}{x^2}$ ,

where  $k_0$  is the killing power constant and  $x$  is the distance from the source.



If the bacteria are irradiated while at a constant distance  $x$  from the light source, this equation may be integrated to give:

$$\log_{10} \frac{N_0}{N_t} = 0.4343 \frac{k_0}{x^2} t \quad (2)$$

where  $N_0$  is the number of bacteria in a unit volume at time zero,  $N_t$  is the number remaining at time  $t$ , and the factor 0.4343 is introduced when

the logarithm to the base 10 is used. The quantity  $\log_{10} \frac{N_0}{N_t}$  is the reduc-

tion and will be designated by  $F$ . The actual reduction in the number of bacteria present may be expressed in several ways as shown in table 1.

TABLE 1. *Reduction factors*

| Number starting | Number remaining | Percentage reduction | Percentage remaining | Reduction factor $F$ |
|-----------------|------------------|----------------------|----------------------|----------------------|
| 10              | 1                | 90                   | 10                   | 1                    |
| 100             | 1                | 99                   | 1.                   | 2                    |
| 1000            | 1                | 99.9                 | 0.1                  | 3                    |
| 10000           | 1                | 99.99                | 0.01                 | 4                    |
| 100000          | 1                | 99.999               | 0.001                | 5                    |

Equation (2) may be simplified by substituting the reduction factor  $F$  to give:

$$t = \frac{F x^2}{0.4343 k_0} \quad (3)$$

If contaminated air is moving at a constant velocity  $v$  toward the ultra-violet light source, the velocity required to maintain a certain reduction,  $F$ , between two points at  $x_0$  and  $x_1$  distances from the light may

be found by putting  $dt = -\frac{dx}{v}$  in equation (1) and integrating as be-

fore. This gives:

$$v = 0.434 \frac{k_0}{F} \left( \frac{1}{x_1} - \frac{1}{x_0} \right) \quad (4)$$

If contaminated air is moving past an ultra-violet light source at a velocity  $v$  in a duct of radius  $R$ , the velocity required to maintain a certain reduction  $F$  between points before and after passing the light may be similarly calculated. The mathematics involved are very complicated

however. A very close approximate solution gives the following equation:

$$v = 1.68 \frac{k_0}{FR} \quad (5)$$

It is apparent in these equations that the killing power  $k_0$  of a given lamp with respect to a certain organism is the only term not established in terms of purely physical measurements. The problem therefore is to determine absolute values for  $k_0$  and to determine how  $k_0$  varies with different light sources, for different organisms, and for different air conditions.

The development of these equations does not include the effect of reflection from the inside of the irradiation duct upon the value of  $k_0$ . The inside of this duct was painted to give it a minimum reflectivity. Nevertheless, the small percentage of reflection will cause a large increase in intensity of radiations at any point. The formulation is unaffected by this change for the intensity is increased by a constant ratio at any point in the duct. The result is that the values of killing power are somewhat higher than those which would be obtained with no reflection, but that they are lower than those values which would be obtained with an ordinary unpainted galvanized duct.

It is obvious that these equations have been built up from purely theoretical considerations and it remains for the experimental work to establish their validity. For each set of experimental conditions a  $k_0$  was calculated from equation (4). The  $k_0$  as used was the mean of all ten constants which it was possible to calculate from the ten possible combinations of samples taken from the five sampling points.

## RESULTS

The samples taken during this investigation gave the numerous data necessary to draw accurate conclusions where biological reactions are involved. These results will be discussed under the following topics:

- a. The effect of variations in lamp voltage upon killing power.
  - b. The effect of humidity upon killing power.
  - c. The effect of light intensity upon killing power.
  - d. Killing effected between sampling points, approaching the light.
  - e. Comparison of killing power of various ultra-violet light sources.
  - f. Effect of light filters upon the killing power of the ultra-violet light source.
  - g. Relative susceptibility of different organisms to ultra-violet light.
- Parts a. through f. were studied using *Escherichia coli* as the test organism.

### EFFECT OF LAMP VOLTAGE

Early in the experimental work it was noticed that variations in operating characteristics of the Uviarc caused great variations in the killing power. This effect was investigated by varying the velocity of air flow

past the light, thus changing its temperature, or by varying the external resistance in series with the arc, all other factors remaining constant. The resulting values of  $k_0$  were calculated and plotted against various com-

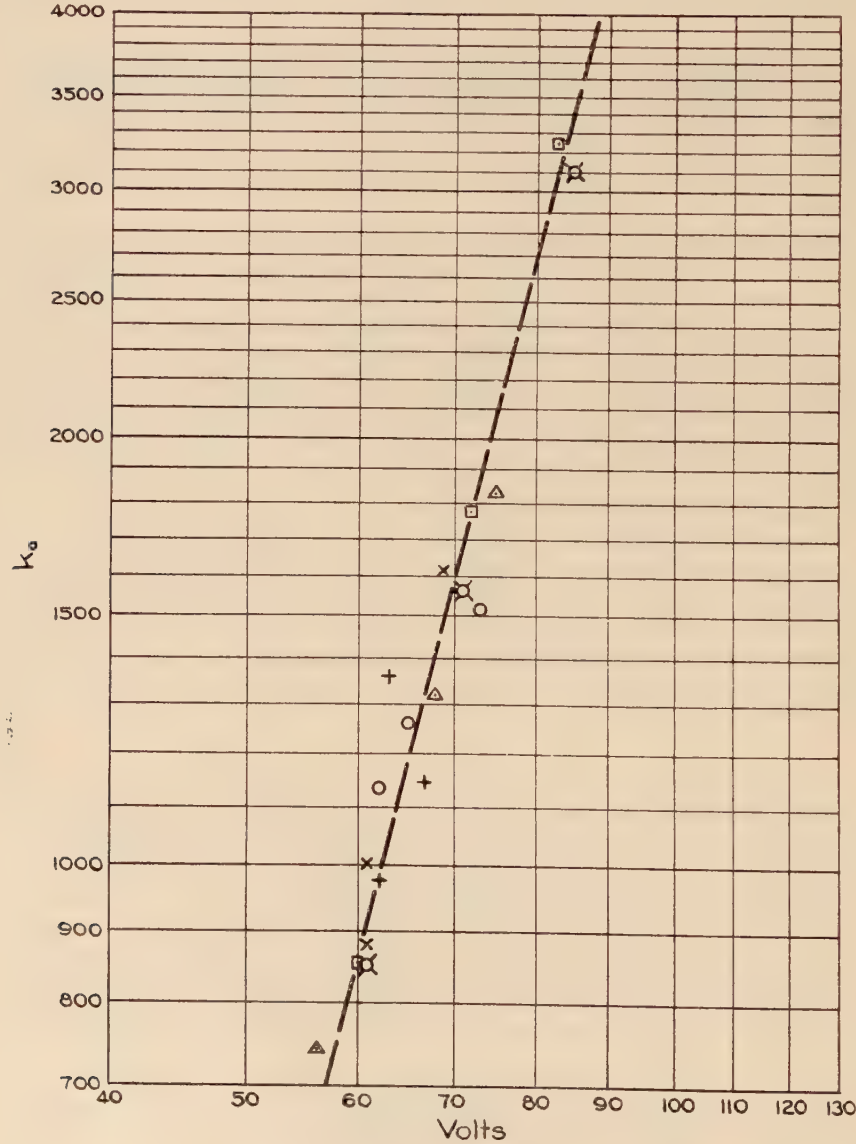


FIG. 2. Relation between  $k_0$  and voltage across arc.

binations of voltage, current, power, etc. The only consistent plot was that between  $k_0$  and voltage as shown in figure 2. The slope of the line indicates that the killing power varies as the fourth power of the voltage

drop across the arc which was to be expected. This may be but a masking of other fundamental factors, but nevertheless serves as an excellent empirical means of correcting  $k_0$  for variations in the operating characteristics of the arc.

#### HUMIDITY EFFECT

It was found that humidity has a very important effect upon the killing power of ultra-violet radiations. Plotted in figures 3 and 4 are 54 points obtained with the Uviarc and *Escherichia coli*. In figure 3,  $k_0$  is

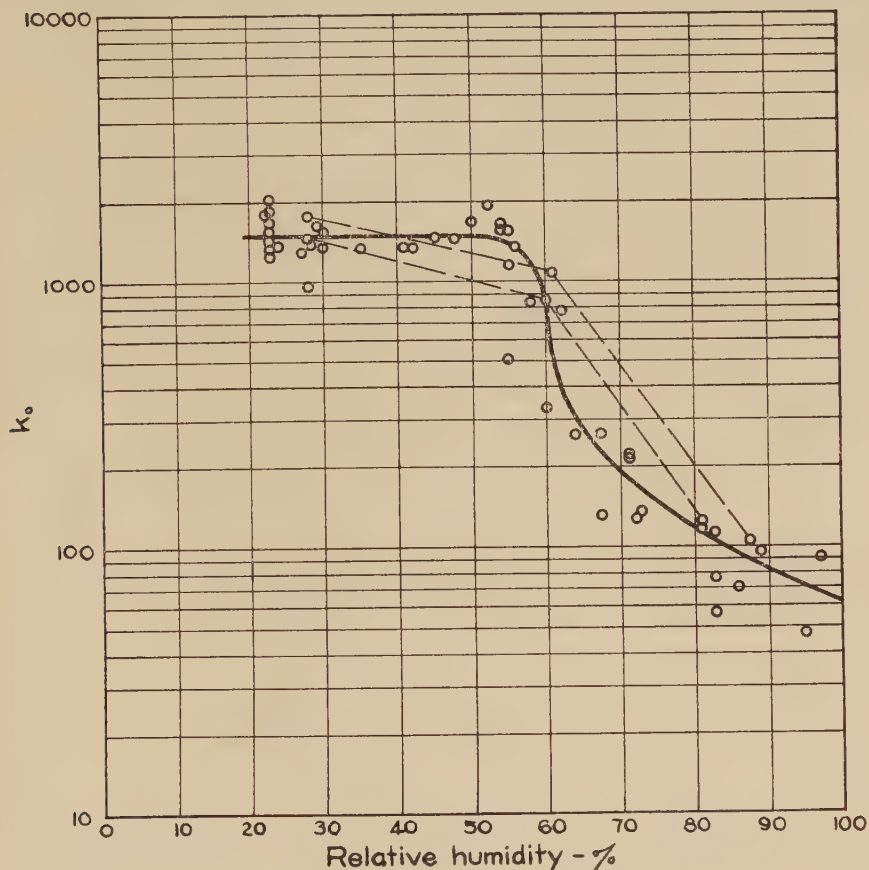


FIG. 3. Relation between relative humidity and  $k_0$  for Uviarc and *Escherichia coli*.

plotted against relative humidity and in figure 4,  $k_0$  is plotted against absolute humidity. As most points were obtained with air at about the same dry bulb temperature, both plots tend to be similar and consequently no conclusion as to which humidity is the controlling factor could be made. However, two runs of three points each were obtained at very low dry bulb temperatures. The three points obtained in each of these two



runs are connected by dashed lines in both plots. The fact that these points coincide with all the other points in the plot against relative humidity, but that they are far out of line in the plot against absolute humidity indicates definitely that the relative humidity is the factor directly affecting the killing power.

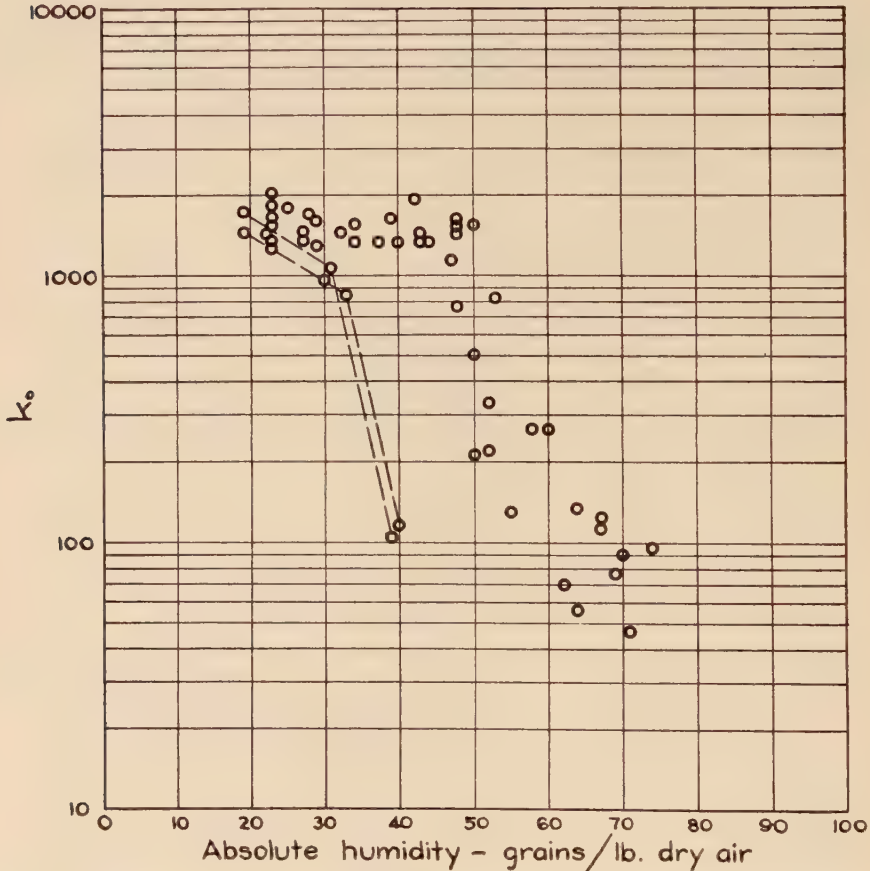


FIG. 4. Relation between absolute humidity and  $k_0$  for Uviarc and *Escherichia coli*.

The plot of  $k_0$  against relative humidity indicates that the killing power of ultra-violet radiations upon bacteria in air is unaffected by humidities below about 55 per cent R. H. Above that point there is a sharp break so that at 65 per cent R. H. the killing power is but one-fifth as great as it is in dry air, at 75 per cent it is one-tenth as great and at 100 per cent it is but one-twenty-fifth as great. This indicates that at the critical point some new factor enters the situation. The extreme sharpness of the break would indicate that this factor is physical rather than biological. The increase in humidity may cause a change in the surface conditions of either the particle containing the bacterium being irradi-

ated or the organism itself, which by refraction or reflection prevents a large part of the radiations from penetrating the organism. According to Lyman<sup>4</sup> and also to Anderson and Robinson<sup>5</sup> water is quite transparent to ultra-violet in the spectrum range of the quartz mercury arc so that absorption of the rays by a thin water layer could not furnish an explanation of the observed variations.

#### EFFECT OF INTENSITY OF RADIATIONS

The low pressure mercury arc provided a convenient means of studying the effect of variations in intensity of the radiations upon the killing power of the arc. The total intensity of the radiations could be

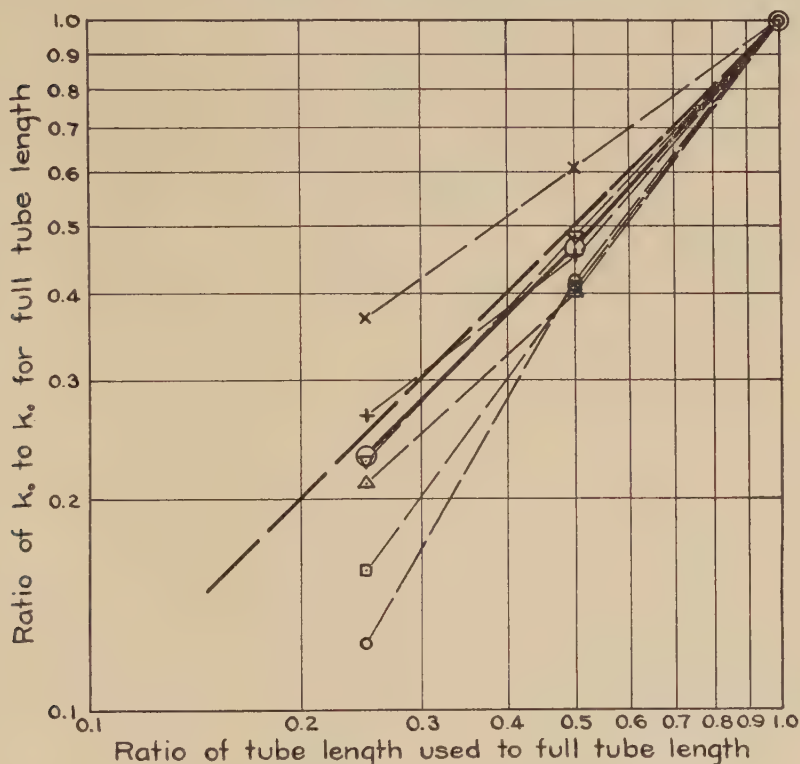


FIG. 5. Relation between  $k_0$  and intensity.

varied by shielding different lengths of the quartz tube. In actual use 3-inch, 6-inch and 12-inch lengths of the tube were used and  $k_0$ 's obtained for each.

Using the  $k_0$  obtained with the 12-inch tube as a base, the ratios of the  $k_0$ 's obtained with the other lengths were calculated and plotted against the proportion of full tube length exposed. The plot of these values for the several runs is shown in figure 5. The mean of all values

is shown by the large circles connected by the full line. The heavy dashed line is drawn at a 1:1 slope and is included within the range of the standard deviation of the mean of all points. It must be concluded, therefore that within the limits of experimental error the killing power is directly proportional to the intensity of the radiations.

#### KILLING BETWEEN SAMPLING POINTS

The values of  $k_0$  as calculated for the ten possible combinations of pairs for the five sampling points were used as a means of checking the validity of the equations already developed. As the intensity of the radiations varies as 256 to 1 throughout the length of the irradiation duct, a  $k_0$  which remains constant throughout its length would be an excellent check of the fundamental assumptions.

The runs used for this analysis were divided into two groups, one connecting 16 sets of observations below 50 per cent relative humidity and the other containing 20 sets of observations above 70 per cent relative humidity. For each set the mean value of  $k_0$  was determined and the ratio of the ten individual values to this mean was determined. The mean value and the standard deviation of all ratios calculated between the same points were determined.

Equation (4) may be written as follows:

$$F = \log \frac{N_0}{N_t} = 0.434 \frac{k_0}{v} \left( \frac{1}{x_1} - \frac{1}{x_0} \right)$$

From this it is seen that  $\log \frac{N_0}{N_t}$  will plot as a straight line against  $\frac{1}{x}$  on

arithmetic paper or  $\frac{N_0}{N_t}$  will plot as a straight line against  $\frac{1}{x}$  on semi-log

paper, providing  $v$  is kept constant, in which case the slope of the curve is a direct function of  $k_0$ . Assuming  $v = 100$  feet per minute, such a plot was made for the actual values of  $k_0$  determined between sampling points at relative humidities below 50 per cent. This is shown in figure 6. Similarly, figure 7 is a plot for the  $k_0$ s determined with relative humidities above 70 per cent, but with  $v=50$  feet per minute.

It may be seen that the slope of the curve, and consequently the calculated values of  $k_0$ , decrease as the light source is approached. The actual plotted points, therefore, deviate from the theoretical straight line. The most reasonable explanation of this deviation is that the organisms themselves and the particles in which they are suspended are not identical. That such a situation would cause a curve of this type may be shown by assuming that the bacteria are of two homogeneous types each of which would die as shown by the straight, dashed lines in the plot. If the number of organisms of each kind remaining at any point be added together a curve practically identical with that actually obtained will re-

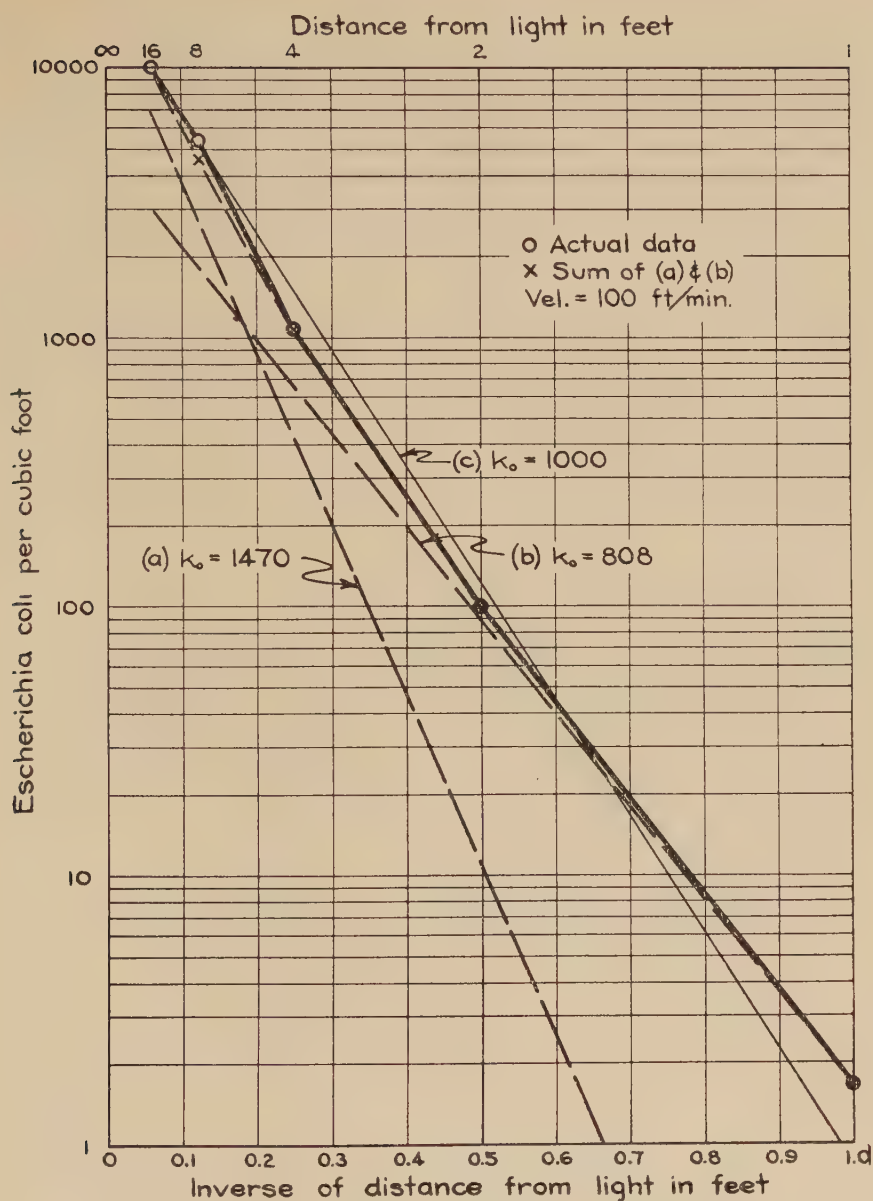


FIG. 6. Death of *Escherichia coli* approaching light in dry air.

sult. That a combination of organisms of differing characteristics is present is only logical under the conditions. It is apparent from the two diagrams that the range of variation is much greater in the humid air than in the dry air, as might be expected.



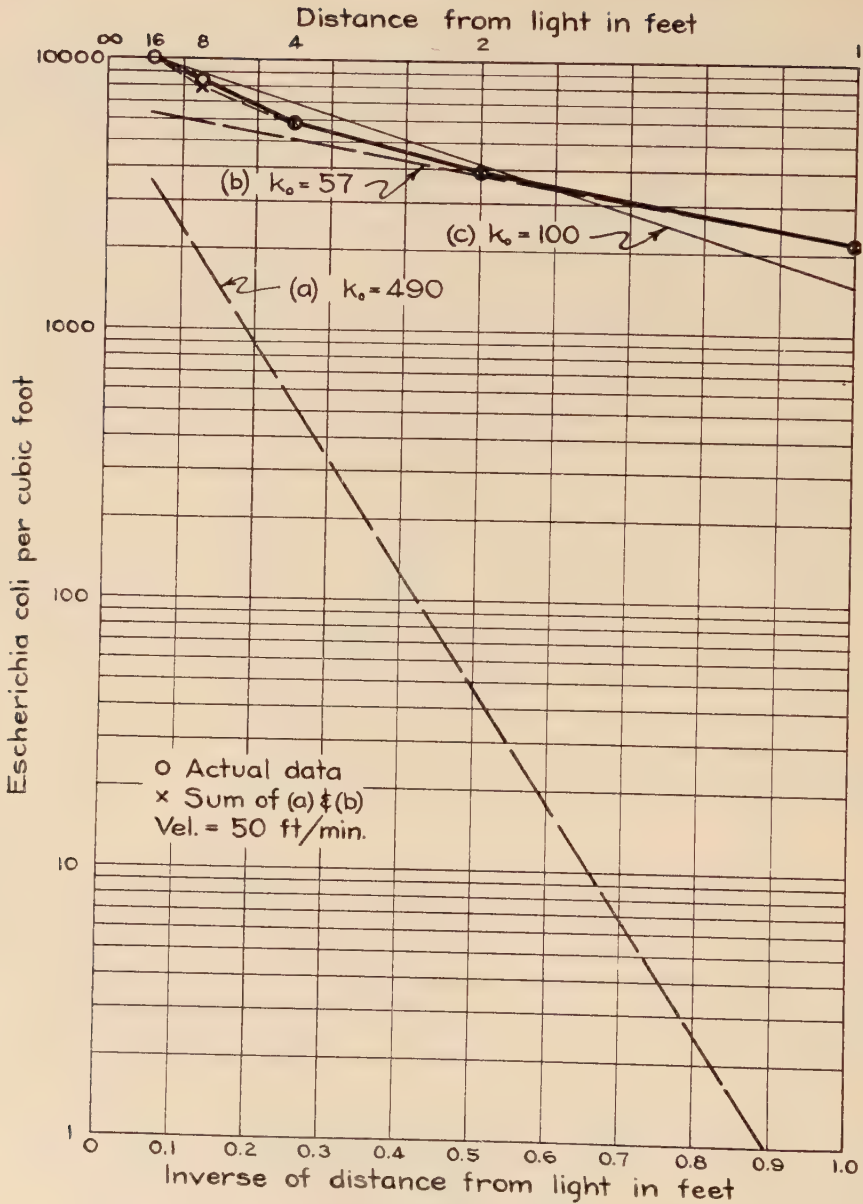


Fig. 7. Death of *Escherichia coli* approaching light in humid air.

If the observed results had plotted as a straight line, the experimental results would have completely justified the formula used and with it the original assumptions. They did not, but the comparatively small deviation may be accounted for by modifying the assumption that all the organisms are identical. This modification in no way impairs the mathematical valid-

ity of the original equations but merely recognizes the fact that it was impossible to reach ideal uniformity with the experimental methods available. If this modification is valid, the curve agrees with the mathematical equations. It should also be noted that the variation of killing effect from a straight line on semi-logarithmic paper is a common observation in studies of disinfection and natural bacterial death rates.

#### KILLING POWER OF DIFFERENT LIGHT SOURCES

The four different ultra-violet light sources gave widely different values for the killing power. Table 2 shows the actual killing powers of the various light sources at several values of relative humidity. On the basis of equal power consumption it is observed that the low pressure arc is 1.6 times more efficient than the Uviarc in dry air and 3 times more efficient in humid air. The Hanovia Alpine is about half as efficient as the Uviarc. The carbon arcs tested were extremely inefficient bactericidally, the Uviarc being 30 times as efficient as the carbon arc using plain carbons. The use of therapeutic carbons increased the efficiency of the carbon arc about 50 per cent.

#### EFFECT OF LIGHT FILTERS

Glass filters placed between the light source and the bacteria have the effect of removing certain portions of the spectrum. In this work Corex D and Pyrex filters were used. The Corex D transmits 71 per cent of the incident energy at 302 m $\mu$ , 9 per cent at 266 m $\mu$ , the point of maximum efficiency of killing, and practically none below 250 m $\mu$ . The Pyrex transmits 13 per cent at 302 m $\mu$  and none below 285 m $\mu$ .

The Corex D filter used with the Uviarc reduced the value of  $k_0$  in dry

TABLE 2. *Bactericidal power of various light sources*

| Source                                | Volts | Watts | Value of $k_0$ at<br>relative humidity of |                |                |                |
|---------------------------------------|-------|-------|---|----------------|----------------|----------------|
|                                       |       |       | 22<br>per cent                            | 35<br>per cent | 68<br>per cent | 90<br>per cent |
| Uviarc                                | 70    | 300   | 1500                                      | 1500           | 210            | 74             |
| 14 in. Low<br>Pressure<br>Mercury Arc | 30    | 105   | 800                                       | 920            | 190            | 95             |
| Hanovia Alpine                        | 40    | 172   | 289                                       |                | 59             |                |
| Hanovia Alpine                        | 46    | 184   | 444                                       |                | 102            |                |
| Carbon Arc<br>Plain                   | 75    | 949   |   | 106            |                |                |
| Plain                                 | 75    | 1050  |   | 175            |                |                |
| Therapeutic                           | 80    | 1120  | 230                                       |                |                |                |

Note: *Escherichia coli* used as test organism.

air from 1500 to 92. At 75 per cent relative humidity the  $k_0$  was reduced from 150 to 38. The Pyrex filter gave values of 51 at 25 per cent relative humidity and 15 at 71 per cent relative humidity under similar conditions. The fact that the filters reduce the killing power of a quartz mercury arc to six per cent or less of its killing power when bare is abundant evidence that for practical purposes the radiations below 280  $m\mu$  are the important bactericidal agents.

#### SUSCEPTIBILITY OF DIFFERENT ORGANISMS

As pointed out previously, the killing power,  $k_0$ , is a factor which depends not only upon the source of radiation, but also upon the type of organisms irradiated. The results of the irradiation of four dissimilar organisms by the same low pressure ultra-violet light source are shown in table 3. Although this information was obtained from a few individual runs and is not backed by numerous checks, it is of some interest.

TABLE 3. Killing effect of ultra-violet radiations upon various bacteria

| Name                                      | Value of $k_0$ at<br>relative humidity of |                |                |                |
|---|---|----------------|----------------|----------------|
|   | 30<br>per cent                            | 52<br>per cent | 59<br>per cent | 85<br>per cent |
| <i>Escherichia coli</i>                   | 880                                       | 1000           | 600            | 100            |
| <i>Sarcina lutea</i>                      |   | 12             |                |                |
| <i>Staphylococcus aureus</i>              | 269                                       |                |                | 87             |
| <i>Bacillus subtilis</i><br>2 day culture |   |                | 160            | 76             |
| 4 day culture                             |   |                | 113            |                |

Note: All values obtained with low pressure mercury arc.

The normal air-borne organisms *Sarcina lutea*, *Staphylococcus aureus*, and *Bacillus subtilis* are considerably more resistant to ultra-violet radiations than is *Escherichia coli*. The variations between the air-borne organisms themselves may be accounted for by differences in their grouping. For instance, the tendency of *Sarcina lutea* to form large tenaceous packets in which the outer cells protect the inner ones may account for the fact that it is 10 to 20 times more resistant than the other air-borne bacteria tested. The 48-hour culture of *Bacillus subtilis* contained numerous spores. The results indicate that the spores are no more resistant and perhaps less so than the cells in the vegetative state.

Another factor which should be considered is that the organisms which form in packets will form droplet nuclei which will be infected by more than one bacterial cell. As the sampling method is really one of determining the number of infected nuclei present, not individual organisms, the ultra-violet light must kill every cell in the nucleus before a reduction is indicated. Thus, although individual cells might be killed as readily,

bacteria which form clusters of cells of any type may appear to be more resistant than bacteria which form discrete cells.

The air-borne organisms show an increase in resistance at high humidities as do *Escherichia coli*. However, the ratio of increase is not so great, indicating that the change in resistance with changes in humidity is a function of the cell itself. The humidity appears to affect different bacteria to varying extents.

#### ENGINEERING APPLICATIONS

The equations previously stated appear to describe adequately the effect of ultra-violet radiations upon bacteria suspended in air. The engineer may apply them directly to the problem of irradiation of a room or disinfection of air passing through a cylindrical ventilation duct.

The simplest problem is that of the sterilization of the air in a room. If an ultra-violet light be suspended in the room equation (3) applies:

$$t = \frac{F x^2}{0.4343 k_0} \quad (3)$$

A desired reduction ratio of  $\frac{N_0}{N_1}$  to be accomplished at distance  $x$  in time

$t$  is chosen and  $k_0$  is calculated. The value of  $k_0$  determines the number of a particular type of lamp which are necessary to produce the required killing of the bacteria. The formula may be used in constructing a simple chart as shown in figure 8 from which the values of  $k_0$  may be taken directly.

In sterilizing the air passing through a ventilation duct by placing the ultra-violet light in the duct, equation (5) may be used. For convenience it may be expressed in terms of the quantity  $Q$  of air being irradiated in a unit of time, where

$$Q = 5.28 \frac{k_0 R}{F}$$

Assuming as before the appropriate values of  $F$ ,  $Q$ , and  $R$ ,  $k_0$  may be calculated and the number of lights required to produce the desired result may be determined.

The relative simplicity of these equations recommends them for practical uses. The only experimental information necessary is the value of the constant for the particular lamp and organism under consideration.

#### CONCLUSIONS

The following general conclusions seem warranted by the experimental work.

1. The bacterial killing power of ultra-violet radiations varies directly as the time of exposure of the bacteria to the light.



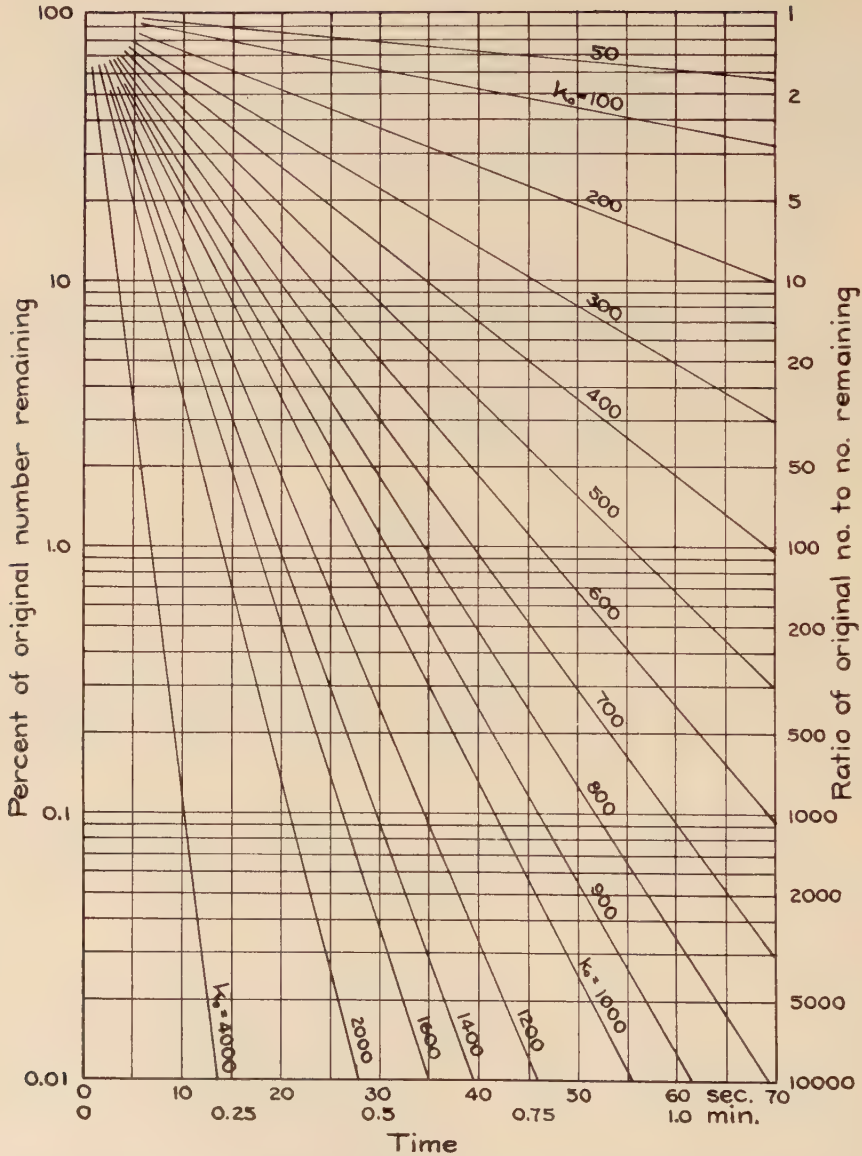


FIG. 8. Generalized diagram of killing of bacteria by U. V. radiations from source 10 feet away.

2. The bacterial killing power of ultra-violet radiations varies directly as the intensity of the light.

3. The bactericidal effect of ultra-violet radiations may be expressed in the form of simple mathematical relationships.

4. The killing effect is remarkably dependent upon the relative

humidity of the air, the ultra-violet radiations being 10 or more times as effective in dry air as in humid air.

5. The use of glass filters indicates that over 94 per cent of the killing effect of the quartz mercury arc lies in the region of the spectrum below 280 m $\mu$ .

6. For a given high pressure quartz mercury arc the killing power varies as the fourth power of the voltage drop across the lamp.

7. On the basis of power consumption, the low pressure mercury arc is 60 per cent more efficient as a bactericidal agent than the 110 V. Uviarc, a high pressure unit. The Hanovia Alpine is one half as efficient bactericidally as is the 110 V. Uviarc. The efficiency of the carbon arc for this purpose is but a few per cent of that of the mercury arc.

8. The value of the killing power,  $k_0$ , expressed in feet and minutes, was 1500 for a 110 V. Uviarc and *Escherichia coli* in air at less than 55 per cent relative humidity.

9. Between different genera of bacteria there are great variations in resistance to ultra-violet radiations. *Sarcina lutea*, a normal air-borne organism, was observed to be 100 times more resistant than *Escherichia coli*, a normal inhabitant of the intestinal tract of man. The other air-borne organisms studied, *Staphylococcus aureus* and *Bacillus subtilis*, were 3 and 8 times more resistant than *Escherichia coli*, respectively.

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## ANAEROBIC DECOMPOSITION AND GASIFICATION OF CORNSTALKS BY THERMOPHILES

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Received December 26, 1939

A great deal of interest is being shown in the utilization of farm wastes, particularly those of a fibrous nature, such as cornstalks and cereal straws. The fermentation of these raw materials had been suggested as one possible method of attacking the problem. With this object in mind the authors initiated a series of experimental studies to ascertain the feasibility of fermenting such farm wastes. It must be emphasized that these fibrous farm products are of complex nature and consist of cellulose, hemicellulose, lignin, and probably some other constituents as yet unidentified. Any practical use that can be made of any of these individual constituents should contribute toward the solution of the problem.

One possibility which suggested itself was the production of fuel gas from these wastes. Recent studies by the authors (21) have shown that the rate of gas production by the anaerobic fermentation of farm wastes was greater at 50° to 55°C. than at 28° to 30°C., although the total amount of gas produced was about the same. It was ascertained also that the fineness of the material and its physical make-up played an important role in the susceptibility of the fibrous material to anaerobic microbial decomposition. In these previous studies no attempt was made at periodic chemical analyses of the residues, since the main object was to determine how much utilizable gas could be produced from a unit quantity of waste material. The next logical step was to ascertain at what rate the individual constituents were decomposed. This information should be of value in planning experiments for the preliminary treatment of these wastes in the preparation of pulp, in retting, and in composting.

In pursuance of these objectives the authors (22) found that at 28° to 30°C. cornstalk flour was fermented more rapidly than chopped cornstalks and produced more gas in a given unit of time; that in cornstalk flour the cellulose and pentosan losses were considerably greater than in the chopped cornstalks; that the lignin loss was greater in the chopped cornstalks than in the cornstalk flour although this loss was a great deal less than the loss of either cellulose or pentosans. In the above study, particular emphasis was placed on the determination of the rate of loss of cellulose, pentosan, and lignin in relation to the rate and total amount

<sup>1</sup> Established by the Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture, in cooperation with the Iowa State College, Ames, Iowa.



of gas produced. The fermentation gases produced were analyzed quantitatively to determine empirically their possible industrial value.

The study herein reported is a companion paper to the previous publication by the authors (22) and deals with a similar study at 50° to 55°C.

The authors are not aware of any previous work dealing with a study of the decomposition and gasification of fibrous farm wastes by a thermophilic methane-producing seed. However, a great deal of work has been done with thermophilic micro-organisms on various products and some of the principles involved are indirectly connected with the work herein reported. The existence of thermophilic micro-organisms in soil, feces, water, milk, and other natural substrates was first definitely established by Miquel in 1879 (20). His work was followed by numerous other investigators (2, 14, 15, 19, 23, 27). Some investigators have been particularly interested in the fermentation of cellulose by thermophiles (16, 17, 24, 25, 28), some in the digestion of sewage solids (1, 5, 6, 8, 9, 10, 11, 12, 13, 18, 26), some in the production of artificial manure (7), and others (3) in the production of power and fuel gas.

#### EXPERIMENTAL

The preparation of the materials, the technic employed, and the analytical methods used were identical with those outlined in a previous publication (22). In the work herein reported a thermophilic methane-producing seed, originally developed from packing-house waste, was employed. The temperature of incubation was 50° to 55°C.

For convenience the study is divided into the following three phases:

1. Anaerobic decomposition of chopped and ground cornstalks submerged in an active methane-producing seed.
2. Losses caused by screening and washing the residues remaining from the anaerobic fermentation of chopped cornstalks.
3. Anaerobic decomposition of chopped and of ground cornstalks submerged in water.

#### RESULTS AND DISCUSSION

1. *Anaerobic decomposition of chopped and of ground cornstalks submerged in an active methane-producing seed.*

In table 1 and figure 1 are presented the cumulative amounts of gas evolved. Gas was produced more rapidly from the cornstalk flour (200 mesh) than from the chopped cornstalks (¼-inch); the respective volumes, per gram of volatile solids (loss on ignition) added, were 508 and 355 cc. after 10 days of incubation. The average composition of gas produced from the two materials was not significantly different, being for the cornstalk flour, carbon dioxide 37.5 per cent, hydrogen 1.3 per cent, and methane 58.8 per cent, and for the chopped cornstalks, carbon dioxide 35.9 per cent, hydrogen 1.5 per cent, and methane 56.6 per cent.

TABLE 1. *Gas production from cornstalks<sup>1</sup> at 50°-55°C.*

| Fermentation period in days                | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cornstalk flour <sup>2</sup>               |     |     |     |     |     |     |     |     |     |     |
| cc. of gas* per gram vol. solids added ..  | 105 | 235 | 335 | 378 | 401 | 443 | 480 | 491 | 498 | 508 |
| pH .....                                   | —   | 7.2 | —   | 7.2 | —   | 7.1 | —   | 7.1 | —   | 7.1 |
| Chopped cornstalks <sup>3</sup>            |     |     |     |     |     |     |     |     |     |     |
| cc of gas* per gram vol. solids added .... | 45  | 103 | 165 | 206 | 233 | 263 | 296 | 316 | 337 | 355 |
| pH .....                                   | —   | 7.2 | —   | 7.2 | —   | 7.2 | —   | 7.1 | —   | 7.1 |
| Seed Control                               |     |     |     |     |     |     |     |     |     |     |
| Total gas in cc. ....                      | 35  | 70  | 95  | 140 | 140 | 175 | 205 | 225 | 315 | 315 |
| pH .....                                   | —   | 7.6 | —   | 7.6 | —   | 7.4 | —   | 7.3 | —   | 7.3 |

<sup>1</sup> Corrected to 760 mm. pressure and 60°F.

<sup>2</sup> Cornstalk flour mixture—average gas composition—CO<sub>2</sub> 37.5 per cent; H<sub>2</sub> 1.3 per cent; CH<sub>4</sub> 58.8 per cent.

<sup>3</sup> Chopped cornstalk mixture—average gas composition—CO<sub>2</sub> 35.9 per cent; H<sub>2</sub> 1.5 per cent; CH<sub>4</sub> 56.6 per cent.

\* Cumulative.

Decomposition of the principal constituents (cellulose, pentosans, and lignin) is indicated in table 2 for the cornstalk flour, in table 3 for the chopped cornstalks, and graphically in figures 2 and 3.

The cellulose (pentosan and ash-free) in the cornstalk flour was decomposed more rapidly than that in the chopped stalks, the former showing a loss of approximately 73 per cent and the latter a loss of about 36 per cent after a fermentation period of 10 days.

In the cornstalk flour both the cellulose and pentosans associated with the cellulose were readily decomposed, the respective amounts being 73 and 84 per cent in 10 days. In the case of the chopped cornstalks the pentosans associated with the cellulose were decomposed more rapidly than the cellulose; the respective amounts of decomposition were 86 and 36 per cent in 10 days.

The pentosans in the cornstalk flour were fermented more rapidly than those in the chopped stalks, the respective amounts of decomposition being 78 and 58 per cent in 10 days.

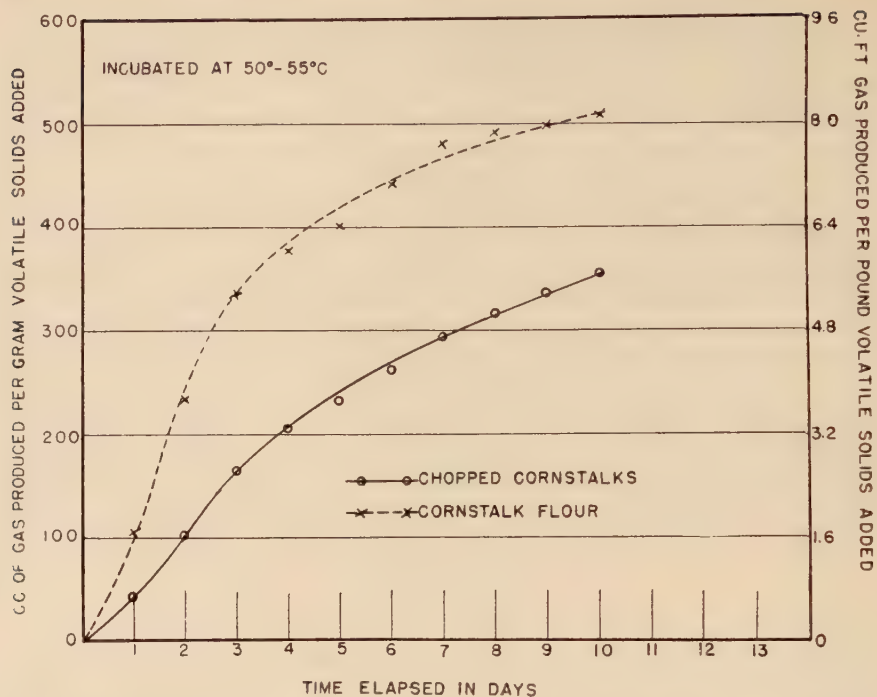


Fig. 1. Gas production from cornstalks submerged in an active methane-producing seed at 50°-55°C.

Although some lignin was decomposed, it was the most resistant of the three principal constituents of the cornstalks. In the cornstalk flour series there was a loss of 17 per cent while in the chopped cornstalk series there was a loss of 33 per cent in 10 days. The reason for the greater decomposition of lignin in the chopped cornstalks is not known. Similar results were obtained at 28° to 30°C. (22).

Boruff and Buswell (4) calculated the theoretical gas yields obtainable from various plant constituents and were able to confirm the results experimentally. Using their theoretical values, a series of calculations, based on the loss of pentosans and cellulose (tables 2 and 3), was made and is presented in table 4. In the cornstalk flour series the ratio of gas produced to the theoretical value calculated from the pentosans and cellulose lost was 1.018:1.000 for the 3-day fermentation period, 1.079:1.000 for the 5-day, and 1.144:1.000 for the 10-day period.

Although the loss of pentosans and cellulose is great enough to account for most of the gas produced during the 3- and 5-day periods, the high recovery may be due to gas produced from constituents other than pentosans and cellulose and the gas produced from the latter two may lag behind initial breakdown owing to the production of intermediates.

TABLE 2. *Decomposition of, and gas production from, cornstalk flour 50°-55° C.*

| Materials added                          |   |                    | After fermenting for |            |           |          |
|--|---|--------------------|----------------------|------------|-----------|----------|
|  | As Seed                                       | As cornstalk flour | Total                | Three days | Five days | Ten days |
| Constituents* present in grams           | Total solids                                  | 34.17              | 23.66                | 57.83      | 46.89     | 45.26    |
|  | Volatile solids                               | 22.65              | 22.42                | 45.07      | 33.96     | 32.30    |
|  | Pentosans                                     | 2.77               | 7.66                 | 10.43      | 5.03      | 4.33     |
|  | Crude cellulose (ash free)                    | 5.51               | 11.39                | 16.81      | 9.72      | 8.72     |
|  | Pentosans in the cellulose                    | 1.23               | 3.37                 | 4.60       | 2.28      | 2.02     |
|  | Cellulose (corrected)                         | 4.28               | 7.93                 | 12.21      | 7.44      | 6.70     |
| Percentage (a) decrease in constituents  | Lignin  | 9.68               | 4.53                 | 14.21      | 13.44     | 13.22    |
|  | Total solids                                  |                    |                      |            | 41.90     | 46.30    |
|  | Volatile solids                               |                    |                      |            | 46.40     | 52.50    |
|  | Pentosans                                     |                    |                      |            | 69.35     | 78.20    |
|  | Crude cellulose (ash free)                    |                    |                      |            | 60.20     | 69.75    |
|  | Pentosans in the cellulose                    |                    |                      |            | 67.90     | 76.00    |
| Gas produced in cc. at 760 mm. and 60°F. | Cellulose (corrected)                         |                    |                      |            | 58.10     | 66.80    |
|  | Lignin  |                    |                      |            | 14.79     | 18.34    |
|  | Total   |                    |                      |            | 8705      | 10520    |
|  | Per gram cornstalk flour added                |                    |                      |            | 368       | 445      |
|  | Per gram vol. solids in cornstalk flour added |                    |                      |            | 388       | 469      |
|  | Per gram vol. solids lost                     |                    |                      |            | 836       | 893      |

(a) Losses noted in seed controls (table 5) were subtracted from total losses in the mixture to obtain losses due to the cornstalk material added

\* Kjeldahl nitrogen determinations were run on all of the samples, but since no extensive accumulation or decrease of nitrogen was noted, these values were not included in this table.



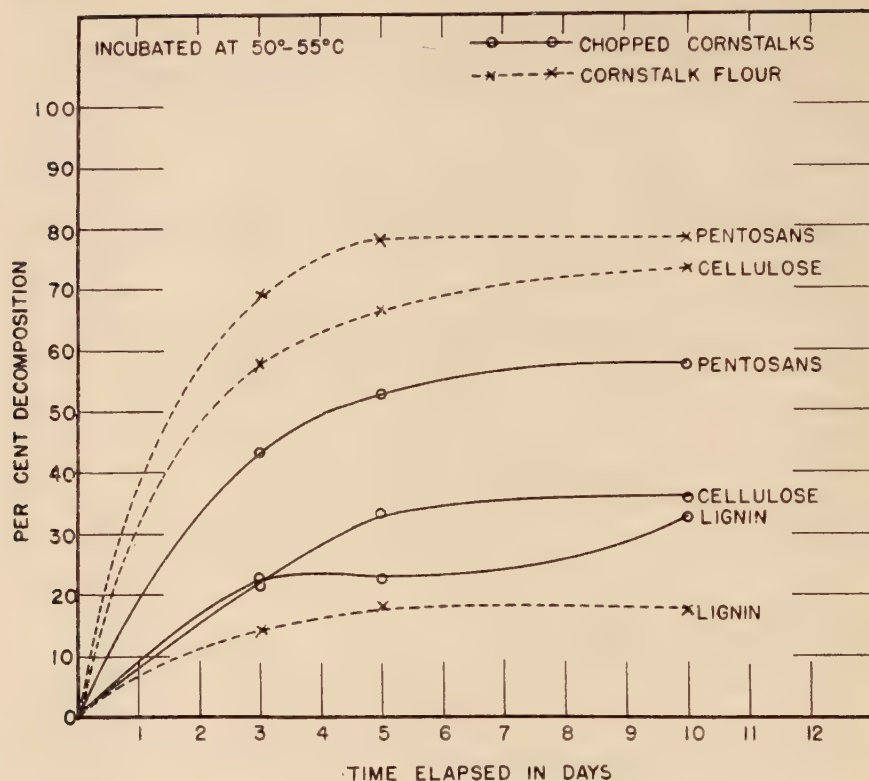


Fig. 2. Breakdown of cornstalk flour and chopped cornstalks submerged in an active methane-producing seed at 50°-55°C.

In the 10-day period, assuming that all the pentosans and cellulose broken down were gasified, practically all the gas produced was accounted for. The additional gas is attributed to the decomposition of organic matter other than cellulose and pentosans.

In the chopped cornstalk series the ratio of gas produced to the theoretical value, calculated from the pentosans and cellulose lost, was 1.205:1.000 for the 3-day fermentation period, 1.230:1.000 for the 5-day, and 1.421:1.000 for the 10-day period. The losses of pentosans and cellulose did not, at any period, account for all the gas produced. Constituents other than pentosans and cellulose may have accounted for the excessive gas. It is possible also that the gas production from the pentosans and cellulose lagged behind initial breakdown owing to the production of intermediates.

The results of the decomposition in the seed controls are presented in table 5. Very little gas was produced and the decomposition of the various constituents was very slow.

Although no actual experimental work was done, the fermentation

TABLE 3. *Decomposition of, and gas production from, chopped cornstalks at 50°–55°C.*

|  | Materials added |                       |       | After fermenting for |           |          |
|--|-----------------|-----------------------|-------|----------------------|-----------|----------|
|  | As Seed         | As chopped cornstalks | Total | Three days           | Five days | Ten days |
| Constituents* present in grams                   |                 |                       |       |                      |           |          |
| Total solids                                     | 34.17           | 24.15                 | 58.32 | 48.50                | 46.45     | 43.40    |
| Volatile solids                                  | 22.65           | 22.35                 | 45.00 | 35.90                | 33.61     | 31.90    |
| Pentosans  | 2.77            | 6.50                  | 9.27  | 6.35                 | 5.71      | 5.14     |
| Crude cellulose (ash free)                       | 5.51            | 11.37                 | 16.88 | 13.15                | 12.24     | 10.98    |
| Pentosans in the cellulose                       | 1.23            | 2.94                  | 4.17  | 2.47                 | 2.58      | 1.63     |
| Cellulose (corrected)                            | 4.28            | 8.43                  | 12.71 | 10.68                | 9.66      | 9.35     |
| Lignin   | 9.68            | 4.49                  | 14.17 | 13.04                | 12.98     | 12.54    |
| Percentage (a) decrease in constituents          |                 |                       |       |                      |           |          |
| Total solids                                     |                 |                       |       | 36.41                | 42.45     | 54.20    |
| Volatile solids                                  |                 |                       |       | 37.60                | 46.60     | 52.60    |
| Pentosans  |                 |                       |       | 43.70                | 53.10     | 57.95    |
| Crude cellulose (ash free)                       |                 |                       |       | 31.50                | 39.03     | 50.70    |
| Pentosans in the cellulose                       |                 |                       |       | 56.80                | 52.40     | 86.45    |
| Cellulose (corrected)                            |                 |                       |       | 22.18                | 33.68     | 35.92    |
| Lignin   |                 |                       |       | 22.93                | 22.93     | 32.70    |
| Gas produced in cc. at 760 mm. and 60°F.         |                 |                       |       |                      |           |          |
| Total  |                 |                       |       | 5020                 | 6835      | 8950     |
| Per gram chopped cornstalks added                |                 |                       |       | 208                  | 283       | 371      |
| Per gram vol. solids in chopped cornstalks added |                 |                       |       | 225                  | 306       | 401      |
| Per gram vol. solids lost                        |                 |                       |       | 597                  | 656       | 761      |

(a) Losses noted in seed controls (table 5) were subtracted from total losses in the mixture to obtain losses due to the cornstalk material added.

\* Kjeldahl nitrogen determinations were run on all of the samples, but since no extensive accumulation or decrease of nitrogen was noted, these values were not included in this table.

Initial volume—1500 cc.

Initial  $\text{NH}_4\text{N}_3$  content—673 p.p.m.

Initial organic  $\text{N}_2$  content—490 p.p.m.

Initial reaction—pH 7.4

$$\text{Ratio} = \frac{\text{vol. solids in stalks}}{\text{vol. solids in seed}} = 0.9980$$

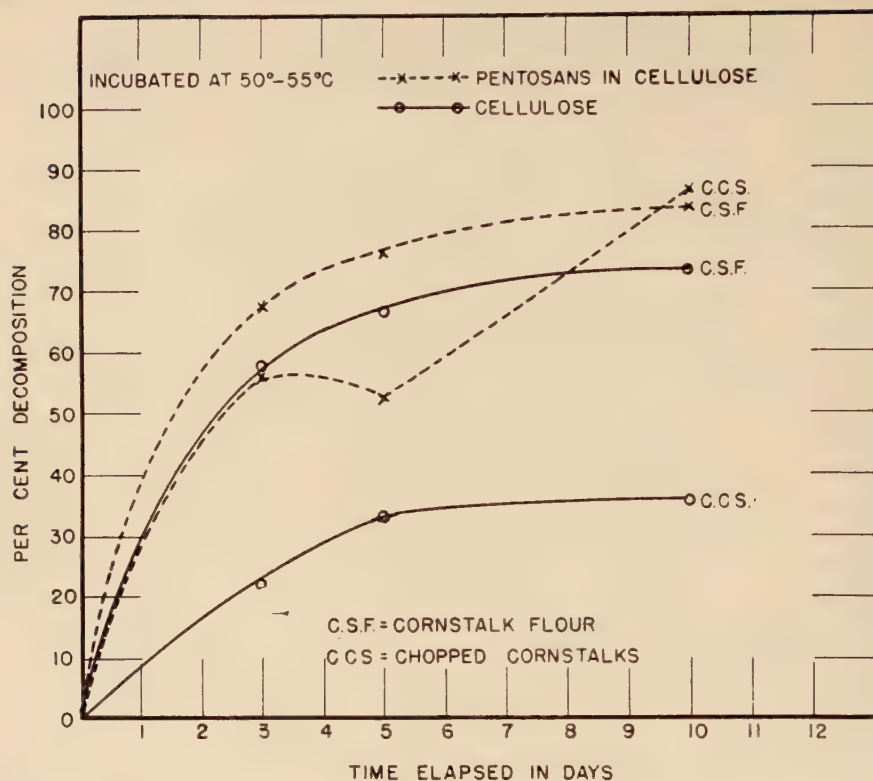


Fig. 3. Decomposition of cellulose and pentosans associated with cellulose in cornstalks submerged in an active methane producing seed at 50°-55°C.

of cornstalk flour, using an active seed, may have possibilities of being useful for the production of a residue having fertilizing properties similar to a compost. In addition, a fuel gas would be produced.

## 2. Losses caused by screening and washing the residues remaining from the anaerobic fermentation of chopped cornstalks.

This study was initiated to determine the percentage loss of constituents caused by the screening and washing of the fermented cornstalks.

A series of samples of chopped cornstalks was prepared and incubated at 50° to 55°C. at the same time and in the same manner as the chopped cornstalk series described in the previous section. This series was an exact duplicate of the previous series, the only difference being that in the first series the entire sample, seed and residue, was evaporated to dryness and analyzed, whereas in this series the seed was screened off (using a 1-mm. round-hole screen) from the chopped stalks and the stalk material washed three times with a total of one liter of

TABLE 4. *A comparison of the total gas produced and of the amounts accounted for (by calculation) from the Cellulose and the Pentosans decomposed*

| Time    | Cornstalk flour* |                |                     |  | Chopped cornstalks*   |                               |                |                |
|---------|------------------|----------------|---------------------|--|---|-------------------------------|----------------|----------------|
|         | Loss in Grams    |                | Gas in cc.          |  | Ratio gas as measured : to  |                               | Loss in Grams  |                |
|         | Pento-<br>sans   | Cellu-<br>lose | As<br>meas-<br>ured | Theoretical<br>yield calcu-<br>lated from<br>cellulose<br>decomposed | Theoretical<br>yield calcu-<br>lated from<br>pentosans and<br>cellulose<br>decomposed | Ratio gas as<br>measured : to | Pento-<br>sans | Cellu-<br>lose |
| 3 days  | 5.40             | 4.77           | 8705                | 8549   | 1.018: 1.000  | 4167                          | 2.92           | 2.03           |
| 5 days  | 6.10             | 5.50           | 10520               | 9750   | 1.079: 1.000  | 5558                          | 3.56           | 3.05           |
| 10 days | 6.43             | 6.14           | 12195               | 10662  | 1.144: 1.000  | 6300                          | 4.13           | 3.36           |

\* 25 grams of material.



distilled water. The washed stalk material was then dried on a steam hot plate, ground to 30-mesh, and analyzed.

The percentage losses of the various constituents in the stalk-seed mixture and in the washed and screened stalk material are presented in tables 3 and 6, respectively, and graphically in figure 4. In the mixture the losses recorded are actual biological decompositions, since the entire

TABLE 5. *Decomposition and gas production in seed control at 50°-55°C.*

|  |                              | Seed<br>at<br>start | After fermenting for |              |             |
|--|------------------------------|---------------------|----------------------|--------------|-------------|
|  |                              |                     | Three*<br>days       | Five<br>days | Ten<br>days |
| Constituents<br>present<br>in<br>grams               | Total solids                 | 34.17               | 33.15                | 32.56        | 32.34       |
|  | Volatile solids              | 22.65               | 21.95                | 21.67        | 21.31       |
|  | Pentosans                    | 2.77                | 2.69                 | 2.66         | 2.34        |
|  | Cellulose                    | 4.28                | 4.12                 | 4.07         | 3.95        |
|  | Lignin                       | 9.68                | 9.58                 | 9.52         | 9.52        |
| Percentage<br>decrease<br>in<br>constituents         | Total solids                 |                     | 2.98                 | 4.71         | 5.36        |
|  | Volatile solids              |                     | 3.09                 | 4.33         | 5.91        |
|  | Pentosans                    |                     | 2.99                 | 3.97         | 15.52       |
|  | Cellulose                    |                     | 3.73                 | 4.90         | 7.71        |
|  | Lignin                       |                     | 1.03                 | 1.65         | 1.65        |
| Gas<br>produced<br>in cc.<br>at 760 mm.<br>and 60°F. | Total                        |                     | 90                   | 225          | 445         |
|  | Per gram vol.<br>solids lost |                     | 129                  | 230          | 332         |

\* Using the initial, 5-days and 10-days values a graph was plotted and the values for the 3-day period were taken from this graph.

mass was analyzed, while in the screened and washed stalk material part of the losses are not true decompositions but are due to the method of preparing the sample for analysis. In this study a procedure was followed that might be applicable in the fermentation of cornstalks for pulp production.

The cellulose loss in the stalk-seed mixture ranged from 22 per cent after the 3rd day of fermentation to 36 per cent after the 10th day, while in the screened and washed materials the loss was 42 and 61 per cent for the same respective periods. It is probable that fine particles of cellulose were liberated from the chopped cornstalks and accumulated in the mixture and subsequently were lost in the screening and washing operations.

The pentosan loss in the seed-mixture was 44 per cent and 58 per

cent, respectively, after 3 and 10 days of fermentation, and 42 and 63 per cent in the screened and washed material for the same time periods.

The lignin loss was 23 and 33 per cent, respectively, after 3 and 10 days of fermentation of the seed-mixture samples. In the screened and washed stalk series the losses for the same respective periods were 31 and 46 per cent. Lignin is not as readily decomposed by micro-organ-

TABLE 6. *Effect of screening and washing the chopped cornstalk residue\**

|  |                       | Initial<br>chopped<br>cornstalks | After fermenting for |              |             |
|--|-----------------------|----------------------------------|----------------------|--------------|-------------|
|  |                       |                                  | Three<br>days        | Five<br>days | Ten<br>days |
| Constituents<br>present<br>in<br>grams       | Total solids          | 24.15                            | 12.63                | 10.18        | 8.61        |
|  | Volatile solids       | 22.35                            | 12.30                | 9.94         | 8.41        |
|  | Pentosans             | 6.50                             | 3.80                 | 3.05         | 2.41        |
|  | Cellulose             | 8.43                             | 4.85                 | 4.02         | 3.28        |
|  | Lignin                | 4.49                             | 3.10                 | 2.66         | 2.42        |
| Percentage<br>decrease<br>in<br>constituents | Total solids          |                                  | 47.70                | 57.80        | 64.40       |
|  | Volatile solids       |                                  | 44.95                | 55.50        | 62.45       |
|  | Pentosans             |                                  | 41.50                | 53.00        | 62.95       |
|  | Cellulose (corrected) |                                  | 42.45                | 52.40        | 61.05       |
|  | Lignin                |                                  | 30.95                | 40.80        | 46.10       |

\* Fermented at 50°-55°C.

isms as are the other cornstalk constituents. In the process of fermentation of the cellulose and pentosans, lignin particles are probably released and accumulate in the mixture. Evidently some of the lignin was lost in the process of screening and washing the fermented stalks. This probably accounts for the larger losses recorded in the screened and washed samples. This fact must be kept in mind in interpretation of data in studies on the fermentation of lignin by micro-organisms to prevent drawing erroneous conclusions.

### 3. *Anaerobic decomposition of chopped and of ground cornstalks submerged in water.*

This study was initiated to determine if an effective fermentation could be induced without the addition of an active seed.

Series of samples of cornstalk flour and of chopped cornstalks were prepared as follows: 25-gram portions of the cornstalk material and 1500 cc. of tap water were placed in two-quart mason jars, which were then closed, sealed, and connected to water seals as in the previous studies. The samples were incubated at 50° to 55°C. for 3-, 5-, and 10-day periods.

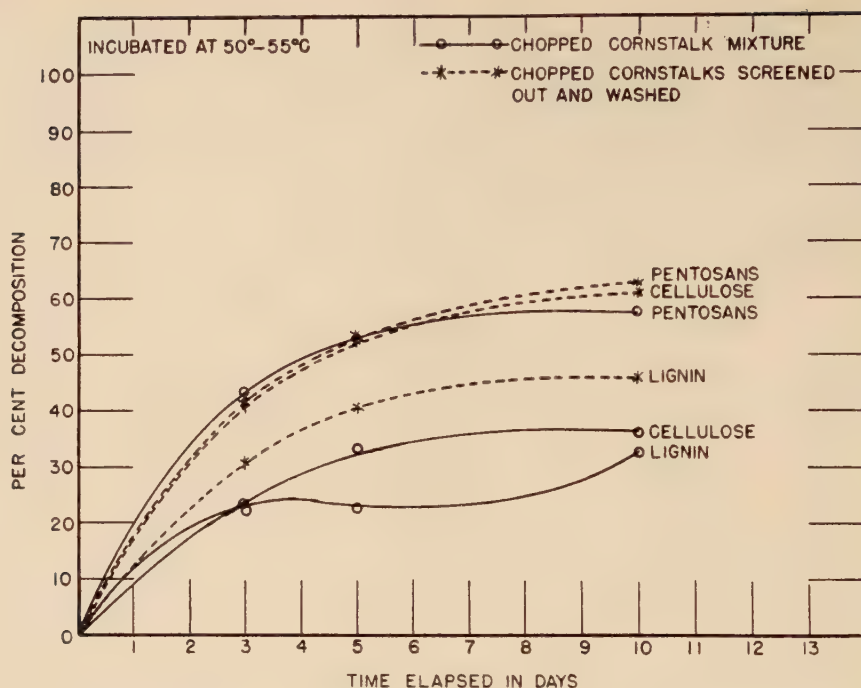


Fig. 4. Effect of screening and washing on the chopped cornstalk residue after fermenting with a prepared seed.

Gas production measurements and pH determinations were made daily. These results are presented in table 7. Very little gas was produced, 16.6 cc. and 20.9 cc. (per gram of volatile solids added), respec-

TABLE 7. Gas production from cornstalks submerged in water (a) at 50°-55°C.

| Fermentation period in days                  | 1   | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
|--|-----|------|------|------|------|------|------|------|------|------|
| Cornstalk flour                              |     |      |      |      |      |      |      |      |      |      |
| cc. of gas* per gram vol. solids added ..... | 6.2 | 10.4 | 10.4 | 12.4 | 12.4 | 12.4 | 14.5 | 14.5 | 14.5 | 16.6 |
| pH .....                                     | 6.2 | 6.1  | 6.2  | 6.0  | 6.0  | 6.1  | 6.0  | 6.0  | 6.0  | 5.7  |
| Chopped Cornstalks                           |     |      |      |      |      |      |      |      |      |      |
| cc. of gas* per gram vol. solids added ..... | 8.4 | 8.4  | 10.6 | 12.7 | 14.5 | 14.5 | 16.7 | 16.7 | 16.7 | 20.9 |
| pH .....                                     | 6.3 | 6.3  | 6.3  | 6.2  | 6.2  | 6.3  | 6.2  | 6.2  | 6.1  | 6.0  |

(a) Corrected to 760 mm. pressure and 60°F.

\* Cumulative

tively, for the cornstalk flour and chopped cornstalks after 10 days incubation. In the cornstalk flour series the pH ranged between 6.0 and 6.2 during the first 9 days dropped to pH 5.7 on the 10th day. In the chopped cornstalk series the pH ranged between 6.0 and 6.3 during the entire 10-day incubation period.

The decomposition of the various constituents is shown in table 8 for the cornstalk flour and in table 9 for the chopped cornstalks, and graphically in figure 5. The amount of breakdown of the various constituents was rather slight in both the flour and chopped stalks.

TABLE 8. *Decomposition of cornstalk flour submerged in water 50°-55°C.*

|  |  | Cornstalk<br>flour<br>used | After fermenting for |              |             |
|--|--|----------------------------|----------------------|--------------|-------------|
|  |  |                            | Three<br>days        | Five<br>days | Ten<br>days |
| Constituents<br>present<br>in<br>grams               | Total solids   | 23.65                      | 23.47                | 22.83        | 22.81       |
|  | Volatile solids  | 21.70                      | 21.69                | 21.45        | 21.40       |
|  | Pentosans  | 7.12                       | 6.93                 | 6.88         | 6.57        |
|  | Cellulose  | 7.94                       | 7.18                 | 7.27         | 7.58        |
|  | Lignin   | 4.42                       | 4.50                 | 4.40         | 4.48        |
| Percentage<br>decrease<br>in<br>constituents         | Total solids   |                            | 0.76                 | 3.47         | 3.55        |
|  | Volatile solids  |                            | 0.01                 | 1.15         | 1.38        |
|  | Pentosans  |                            | 2.66                 | 3.37         | 7.72        |
|  | Cellulose  |                            | 9.57                 | 8.43         | 4.53        |
|  | Lignin   |                            | +1.81                | 0.45         | +1.36       |
| Gas<br>produced<br>in cc.<br>at 760 mm.<br>and 60°F. | Total  |                            | 225                  | 270          | 360         |
|  | Per gram cornstalk flour added                         |                            | 9.5                  | 11.4         | 15.2        |
|  | Per gram volatile solids in corn-<br>stalk flour added |                            | 10.4                 | 12.5         | 16.6        |
|  | Per gram vol. solids lost                              |                            | —                    | 1080         | 1200        |

The extent of decomposition of cellulose was 5 per cent and 11 per cent, respectively, for the cornstalk flour and chopped cornstalks after 10 days of fermentation.

The pentosan decomposition was 8 per cent and 11 per cent, respectively, for the cornstalk flour and chopped cornstalks.

No significant lignin losses were noted. The slight variations noted at times were within the limits of experimental error.

The effect on the losses of the various constituents in the cornstalk flour resulting from filtering off the liquid and washing (at the end of the fermentation period) is shown in table 10 and graphically in figure 6. A slight loss of lignin was noted. The pentosan and cellulose losses were 13 per cent and 10 per cent, respectively.



TABLE 9. *Decomposition of chopped cornstalks submerged in water at 50°-55°C.*

|  |   | Chopped<br>cornstalks<br>used | After fermenting for |              |             |
|--|---|-------------------------------|----------------------|--------------|-------------|
|  |   |                               | Three<br>days        | Five<br>days | Ten<br>days |
| Constituents<br>present<br>in<br>grams               | Total solids  | 24.14                         | 23.07                | 23.05        | 22.24       |
|  | Volatile solids   | 21.32                         | 21.03                | 21.10        | 19.78       |
|  | Pentosans   | 6.50                          | 6.29                 | 6.09         | 5.78        |
|  | Cellulose   | 8.43                          | 7.98                 | 8.03         | 7.51        |
|  | Lignin  | 4.49                          | 4.54                 | 4.49         | 4.48        |
| Percentage<br>decrease<br>in<br>constituents         | Total solids  |                               | 4.43                 | 4.51         | 7.87        |
|  | Volatile solids   |                               | 1.36                 | 1.03         | 7.22        |
|  | Pentosans   |                               | 3.50                 | 6.31         | 11.08       |
|  | Cellulose   |                               | 5.34                 | 4.74         | 10.92       |
|  | Lignin  |                               | +1.10                | 0.00         | 0.22        |
| Gas<br>produced<br>in cc.<br>at 760 mm.<br>and 60°F. | Total   |                               | 225                  | 320          | 455         |
|  | Per gram chopped cornstalks<br>added                      |                               | 9.3                  | 13.3         | 18.8        |
|  | Per gram volatile solids in chop-<br>ped cornstalks added |                               | 10.5                 | 15.0         | 21.4        |
|  | Per gram volatile solids lost                             |                               | 775                  | 1452.0       | 296         |

The effect on the losses of the various constituents in the chopped cornstalks resulting from screening and washing is shown in table 11

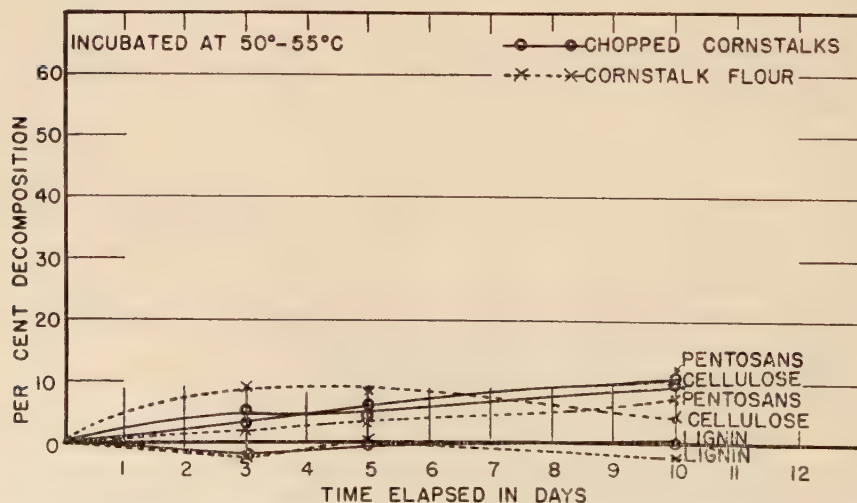


Fig. 5. Breakdown of cornstalk flour and of chopped cornstalks submerged in water at 50°-55°C.

TABLE 10. *The effect of filtering off and of washing the cornstalk flour\**

|  |                 | Cornstalk<br>flour<br>used | After fermenting for |              |             |
|--|-----------------|----------------------------|----------------------|--------------|-------------|
|  |                 |                            | Three<br>days        | Five<br>days | Ten<br>days |
| Constituents<br>present<br>in<br>grams       | Total solids    | 23.65                      | 22.28                | 21.30        | 20.33       |
|  | Volatile solids | 21.70                      | 21.43                | 20.66        | 19.71       |
|  | Pentosans       | 7.12                       | 6.82                 | 6.42         | 6.16        |
|  | Cellulose       | 7.94                       | 7.41                 | 7.06         | 7.11        |
|  | Lignin          | 4.42                       | 4.48                 | 4.32         | 4.34        |
| Percentage<br>decrease<br>in<br>constituents | Total solids    |                            | 5.79                 | 9.93         | 14.02       |
|  | Volatile solids |                            | 1.24                 | 4.79         | 9.19        |
|  | Pentosans       |                            | 4.21                 | 9.82         | 13.49       |
|  | Cellulose       |                            | 6.68                 | 11.08        | 10.47       |
|  | Lignin          |                            | +1.36                | 2.26         | 1.81        |

\* Submerged in water and fermented at 50°-55°C.

+ Plus values indicate increases—probably caused by formation of nitrogen complexes.

and graphically in figure 7. Increased losses in pentosans, cellulose, and lignin due to the screening and washing are indicated. The percentage losses for lignin, cellulose, and pentosans were 20, 19, and 21, respectively.

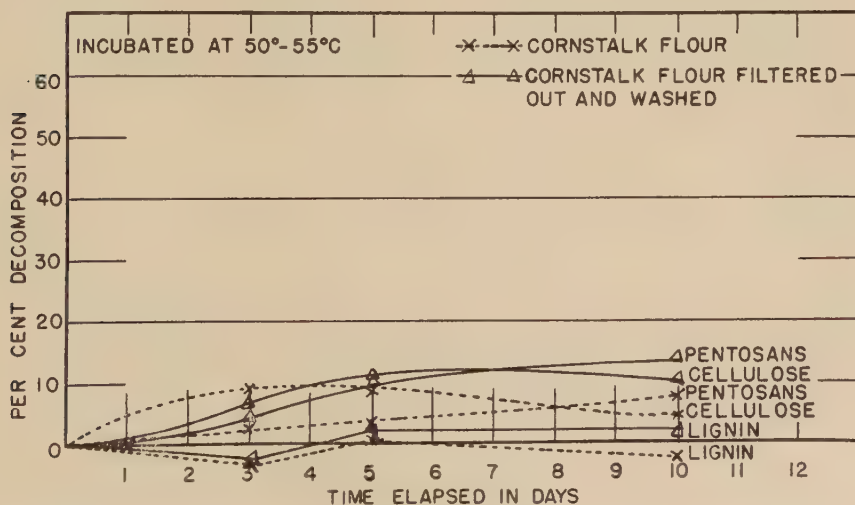


Fig. 6. Effect of filtering and washing on the cornstalk flour residue after submerging in water at 50°-55°C.

TABLE 11. *Effect of screening and washing the chopped cornstalks\**

|  |                 | Chopped<br>cornstalks<br>used | After fermenting for |              |             |
|--|-----------------|-------------------------------|----------------------|--------------|-------------|
|  |                 |                               | Three<br>days        | Five<br>days | Ten<br>days |
| Constituents<br>present<br>in<br>grams       | Total solids    | 24.14                         | 19.36                | 19.71        | 17.71       |
|  | Volatile solids | 21.32                         | 18.89                | 19.01        | 16.71       |
|  | Pentosans       | 6.50                          | 5.73                 | 5.90         | 5.12        |
|  | Cellulose       | 8.43                          | 7.80                 | 7.86         | 6.89        |
|  | Lignin          | 4.49                          | 4.18                 | 4.00         | 3.60        |
| Percentage<br>decrease<br>in<br>constituents | Total solids    |                               | 19.80                | 18.35        | 26.65       |
|  | Volatile solids |                               | 11.40                | 10.88        | 21.65       |
|  | Pentosans       |                               | 11.85                | 9.23         | 21.25       |
|  | Cellulose       |                               | 7.48                 | 6.76         | 18.85       |
|  | Lignin          |                               | 6.91                 | 10.91        | 19.82       |

\* Submerged in water and fermented at 50°-55°C.

#### SUMMARY AND CONCLUSIONS

1. In the anaerobic decomposition at 50° to 55°C., using an active methane-producing seed, cornstalk flour fermented more rapidly than chopped cornstalks, producing 43 per cent more gas in the 10-day period. This gas consisted of 34 to 35 per cent carbon dioxide and 55 to 59 per cent methane. It may be possible to use the methane for production of

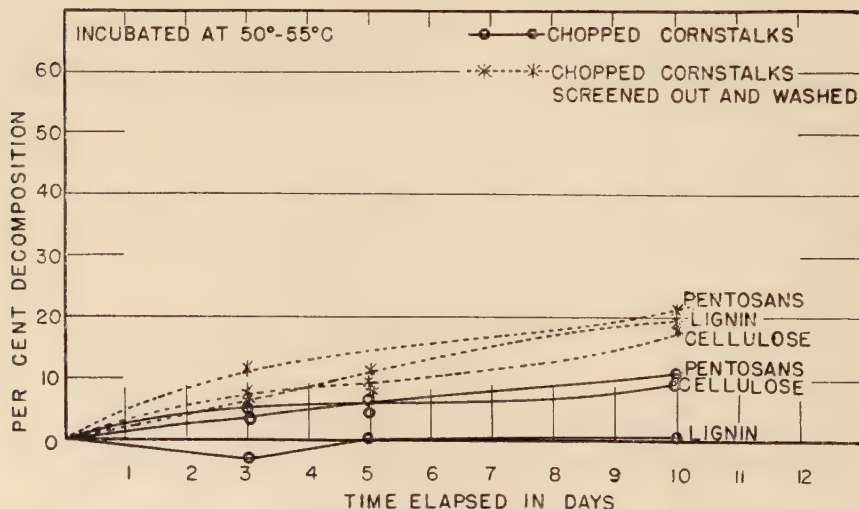


Fig. 7. Effect of screening and washing on the chopped cornstalk residue after fermenting without a prepared seed at 50°-55°C.

heat or power and the carbon dioxide for manufacture of "dry ice" or other products.

2. In the cornstalk flour series about 98 per cent of the gas produced could be accounted for by the cellulose and pentosans decomposed for the 3-day period, 93 per cent for the 5-day period, and 87 per cent for the 10-day period. For the same periods in the chopped cornstalk series 83, 81, and 70 per cent of the gas produced could be accounted for by the cellulose and pentosans decomposed.

3. In the cornstalk flour series the cellulose and pentosan losses were considerably greater than in the chopped cornstalk series. In the latter series the lignin loss was greater than in the cornstalk flour series.

4. By screening and washing the chopped cornstalk residues after fermentation periods of 3 and 5 days, the pentosan losses were not increased. For the 10-day period the losses were slightly increased. For all periods the cellulose and lignin losses were increased by screening and washing.

5. The incubation of cornstalk flour and chopped cornstalks, submerged in water, showed only slight losses of the principal constituents. This procedure offers very little promise for use in pulp manufacture.

6. More work will have to be done on the fermentation of chopped cornstalks, using an active methane seed, before definite conclusions can be drawn regarding its adaptability to the production of pulp.

7. Comparing the results of the anaerobic fermentation of cornstalks at 50° to 55°C. with those at 28° to 30°C. (22), the following points are of particular interest: At the higher temperature the rates of cellulose and pentosan decomposition were higher, the lignin loss was slightly higher, and the rate of gasification was greater, but the total amount of gas produced was about the same as at 28° to 30°C. At 50° to 55°C. in both the cornstalk flour and chopped cornstalks, the pentosans were fermented at a higher rate than was the cellulose; the reverse was true at the lower temperature.

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# THE DISSIMILATION OF PHOSPHOGLYCERIC ACID AND HEXOSEDIPHOSPHATE BY AEROBACTER INDOLOGENES

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Received December 26, 1939

Antoniani (1933) demonstrated that *Escherichia coli* attacks phosphoglyceric acid. Later Tikka (1935) proposed a scheme of dissimilation of glucose in which the Embden-Meyerhof-Parnas reactions for yeast (Meyerhof and Kiessling, 1934) were adapted to *E. coli*. Hexosediphosphate, triosephosphate, α-glycerophosphate and phosphoglyceric acid were included as intermediates. Tikka failed in his attempts to isolate any of the proposed intermediates. All, with the exception of triosephosphate, were shown to be dissimilated by the bacteria. Stone and Werkman (1936) have since reported the isolation of phosphoglyceric acid from *E. coli* and *Aerobacter indologenes* as well as from a number of other bacteria (1937).

Werkman et al. (1937) have shown that *Propionibacterium pentosaceum* will break down phosphoglyceric acid and hexosediphosphate quite rapidly, but prefers glucose under the conditions. Furthermore, these organisms appear to maintain normal growth in 0.02 M sodium fluoride—a concentration sufficient to block almost completely the dissimilation of the phosphate esters used. The authors suggest that the propionic acid bacteria possess at least two mechanisms of glycolysis.

The present investigation undertakes a study of the dissimilation by *E. coli* of two phosphate esters in the presence and absence of glucose; and also to determine the effect of sodium fluoride on the dissimilation.

## EXPERIMENTAL

An experimental setup similar to that described by Werkman et al. (1937) was used to determine the effect of glucose and fluoride on the breakdown of phosphate esters. The fermentations were conducted in large test tubes; the medium consisted of 0.4 per cent yeast extract, 0.5 per cent glucose and a trace of dipotassium phosphate. The solution of phosphoglyceric acid was prepared from the barium salt by precipitation with sodium sulfate; the hexosediphosphate solution was similarly prepared from the calcium salt by precipitation with sodium oxalate. The esters were sterilized by Seitz filtration, and added aseptically to the medium in the fermentation tubes in concentrations approximating 1 per cent. The tubes were inoculated with a dilute suspension of organisms that had been centrifuged from a broth culture and resuspended in distilled water. Each tube received approximately the number of organisms contained in 1 ml. of broth culture, and the volume was



made up to 20 ml. by the addition of sterile water. The cultures were incubated at 30° C., and the course of the fermentation was followed by determining glucose and inorganic phosphate at stated intervals. Glucose was determined by the method of Stiles, Peterson and Fred (1926) and phosphate by the colorimetric method of Kuttner and Lichtenstein (1930). The release of inorganic phosphate to the medium from the phosphate esters was regarded as utilization of the ester for the purpose of this work.

The solutions of phosphate esters used in the experiments for quantitative analysis were prepared as described above. After sterilization by Seitz filtration, the solutions were added aseptically to the autoclaved media. The concentration of the phosphate esters in the solutions used, was estimated by the method of total carbon determination of Osburn and Werkman (1932). Samples were also removed from the fermentation solution immediately after inoculation and at the end of the fermentation to check the organic phosphate content. Inoculations were made with day old growth washed from an agar slant with a few ml. of sterile water.

Carbon dioxide was determined either gravimetrically or volumetrically by collecting in a definite volume of approximately molar standard alkali.

Hydrogen was determined by measuring the volume of gas collected in the bottles which held the alkali for carbon dioxide absorption.

Ethyl alcohol was determined on the neutral volatile fraction of an aliquot of the fermentation liquor by the method of Stahly, Osburn and Werkman (1934). This distillate, obtained by alkaline distillation, must be examined for acetylmethylcarbinol and the proper correction applied to the alcohol value. Each mole of acetylmethylcarbinol yields two moles of acetic acid in the alcohol oxidation.

Volatile acids were determined by acidifying the residue from the neutral volatile distillation to congo red with concentrated  $\text{H}_2\text{SO}_4$ , and collecting twelve volumes of steam distillate from a constant volume of 25 ml. Before acidifying the residue, it was necessary to remove about six volumes with steam to remove all the acetylmethylcarbinol which interfered with the formic acid determination. To avoid interference of hydrofluoric acid in the determination of volatile acids, the distillate was neutralized with calcium hydroxide, evaporated to a low volume and filtered. Calcium fluoride is insoluble and was removed. Acidifying and redistilling gave an acid distillate suitable for the determination of volatile acids. Formic acid was determined by the method of Auerback and Zeglin (1922) and the difference between it and the total volatile acidity represented acetic acid.

Acetylmethylcarbinol was determined by steam distilling a sample of 25 or 50 ml. of the medium at a constant volume of 25 ml. until six volumes of distillate were collected. An aliquot of the distillate was examined for acetylmethylcarbinol by the iodoform titration of Messinger as described by Goodwin (1920) using an ice bath to prevent the alkaline iodine from reacting with ethyl alcohol.

2,3-Butylene glycol was determined by the method of Brockmann and Werkman (1933).

## RESULTS AND DISCUSSION

The results of the dissimilation of phosphoglyceric acid and hexosediphosphate in the presence of glucose and fluoride are shown in table 1. It is apparent the the presence of fluoride markedly diminished the rate of utilization of glucose. On the other hand, the presence of a phosphate ester seemed to exert a stimulating effect in the absence of fluoride. The figures for glucose, when hexosediphosphate is present, are high by an amount approximating 2.5 mg. per ml. at the beginning of the experiment owing to the reducing action of the hexosediphosphate on the reagents used to determine glucose. Phosphoglyceric acid was only slowly utilized as compared to glucose, and the presence of fluoride markedly diminished the rate of utilization. Hexosediphosphate was almost completely utilized, as indicated by the large amount of phosphate liberated, as well as the disappearance of reducing sugar. In the presence of fluoride, however, the utilization of this ester was stikingly inhibited as shown by comparing the inorganic phosphate for the 2 and the 4 day periods. After the utilization of glucose was practically complete (i.e., substantially at 2.5 mg. per ml. due to hexosediphosphate), the hexose ester was attacked, even in the presence of fluoride. After 8 days' incubation, the remaining solution in the tube containing hexosediphosphate and fluoride was analyzed for any phosphoglyceric acid present, using the method outlined previously (Stone and Werkman, 1936). Twenty mg. of the crude barium salt were obtained from about 12 ml. of medium. The salt was characterized by microscopic examination of crystals and solubility in 0.05 N HCL, and alcohol.

Similar experiments carried out in the absence of glucose (table 2) show that phosphoglyceric acid was not appreciably attacked either in the presence or absence of fluoride. There was little growth in these tubes, compared to those containing hexosediphosphate. The hexose ester alone was rapidly dissimilated but as in the previous experiment, fluoride greatly inhibited the breakdown.

In the presence of .02 M NaF, which is enough to block almost completely the kinetics of the Embden-Meyerhof-Parnas scheme, glucose was fermented to final products which are qualitatively the same as in a normal fermentation of glucose (table 3). The yields of carbon dioxide and hydrogen are lower than normal, while the yields of formic and acetic acids are correspondingly increased. This may be the result of the fact that the rate of fermentation in the presence of fluoride, was reduced so that only about 75 per cent of the sugar added was broken down. On the other hand, it may be due to a specific poisoning of the formic dehydrogenase by NaF. Acetic acid, which in the *Aerobacter* fermentation normally displays the characteristics of an intermediate

TABLE 1. *Dissimilation of phosphoglyceric acid and hexosediphosphate in combination with glucose and NaF by A. indologenes*  
 Medium: 0.5 per cent glucose, 0.4 per cent Difco yeast extract  
 Quantities expressed in mg. per ml.

| Time in days | Control |          | NaF 0.02 M |          | PGA concentration |          | PGA NaF 0.02 M |          | HDP     |          | HDP NaF 0.02 M |          |
|--------------|---------|----------|------------|----------|-------------------|----------|----------------|----------|---------|----------|----------------|----------|
|              | Glucose | Inorg. P | Glucose    | Inorg. P | Glucose           | Inorg. P | Glucose        | Inorg. P | Glucose | Inorg. P | Glucose        | Inorg. P |
|              | mg.     | mg.      | mg.        | mg.      | mg.               | mg.      | mg.            | mg.      | mg.     | mg.      | mg.            | mg.      |
| 0            | 5.1     | .075     | 5.0        | .074     | 5.4               | .072     | 5.4            | .067     | 7.4     | .140     | 7.5            | .143     |
| 1/2          | 2.5     | .066     | 4.1        | .069     | 0.7               | .068     | 2.8            | .060     | 2.8     | .289     | 5.3            | .177     |
| 1            | 0.7     | .059     | 4.1        | .065     | 0.3               | .071     | 2.4            | .050     | 2.0     | .69      | 4.8            | .201     |
| 2            | 0.6     | .066     | 3.9        | .061     | 0.6               | .129     | 1.6            | .063     | 0.7     | 1.38     | 4.0            | .210     |
| 4            | 0.3     | .077     | 3.0        | .059     | 0.7               | .314     | 0.3            | .083     | 0.8     | 1.45     | 3.0            | .380     |
| 8            | 0.4     | .075     | 2.7        | .052     | 0.6               | .620     | 0.4            | .310     | 0.3     | 1.40     | 0.9            | 1.050    |
| Total P mg.  |         | .090     |            | .085     |                   | 1.80     |                | 1.81     |         | 1.48     |                | 1.50     |

compound (Reynolds and Werkman, 1936), was also abnormally high with, consequently, a lower yield of 2, 3-butylene glycol.

When the phosphoglyceric acid was fermented in the presence of glucose, increases were noted in carbon dioxide, hydrogen, acetic acid and alcohol. It appeared as though the organisms first attacked the glucose, then slowly proceeded to ferment the phosphoglyceric acid.

Hexosediphosphate was fermented apparently as rapidly as glucose

TABLE 2. Dissimilation of phosphoglyceric acid and hexosediphosphate by *A. indologenes*

Medium: 0.4 per cent Difco yeast extract  
Inorganic phosphate as mg. per ml.

| Time in days         | PGA<br>1 per cent | PGA<br>NaF 0.02 M | HDP<br>1 per cent | HDP<br>NaF 0.02 M |
|----------------------|-------------------|-------------------|-------------------|-------------------|
| 0                    | .067              | .070              | .130              | .144              |
| ½                    | .071              | .072              | .405              | .184              |
| 1                    | .068              | .066              | 1.02              | .201              |
| 2                    | .072              | .085              | 1.36              | .249              |
| 4                    | .103              | .099              | 1.39              | .461              |
| 8                    | .157              | .131              | 1.42              | .731              |
| Total P<br>mg. 1 ml. | 1.84              | 1.86              | 1.46              | 1.48              |

itself. The products were qualitatively the same as those from glucose but some quantitative differences are apparent, especially in 2, 3-butylene glycol, ethyl alcohol and acetic acid, the glycol yield being less than usual, and resulting apparently in an increase in the other two products.

*A. indologenes* prefers glucose to phosphoglyceric acid or hexosediphosphate. When both esters are present most of the sugar is utilized before any marked release of inorganic phosphate occurs. In concentrations of 0.02 M fluoride, the breakdown of glucose is noticeably inhibited; on the other hand, the dissimilation of phosphoglyceric acid and hexosediphosphate was not blocked completely as might be expected. It is quite possible that in the presence of fluoride, the bacterial phosphatases are removing phosphate directly from the esters without involving the intermediary conversion to phosphopyruvic acid. According to Meyerhof and Kiessling, this reaction in undialyzed yeast is almost completely inhibited by 0.02 M fluoride. The fact, that phosphoglyceric acid could be isolated from the fermentation containing the hexose ester, indicates the enzymes of the Embden-Meyerhof-Parnas reactions are present but blocked at phosphoglyceric acid by fluoride. A similar inhibition occurs with the propionic acid bacteria. The ability of *A. indologenes* to grow and produce normal products in the presence of fluoride concentrations as high as 0.04 or even 0.10 molar, presents strong evidence that these organisms do not



TABLE 3. *Dissimilation of glucose, glucose + 0.02 M NaF, glucose + phosphoglyceric acid and hexosediphosphoric acid by A. indologenes*  
 Medium: 2 per cent glucose, 0.1 per cent  $(\text{NH}_4)_2\text{HPO}_4$ , 0.5 per cent  $\text{NaHCO}_3$  volume 300 cc.  
 Products per 100mM of fermented glucose

| Substrate               | $\text{CO}_2$ | $\text{H}_2$ | Acetic acid | Formic acid | Lactic acid | Acetyl-methyl-carbinol | 2, 3-Butylene-glycol | Ethyl-alcohol | mM Phosphoglyceric acid | Final pH | O/R  | Percentage carbon |
|-------------------------|---------------|--------------|-------------|-------------|-------------|------------------------|----------------------|---------------|-------------------------|----------|------|-------------------|
| Glucose                 | 173.00        | 60.05        | 2.74        | 8.70        | 5.68        | 4.15                   | 56.20                | 57.50         |                         | 6.7      | 1.03 | 93.7              |
| Glucose + PGA           | 189.50        | 68.40        | 15.62       | 25.43       | 3.81        | 0.68                   | 57.00                | 76.40         | 23.83                   | 7.0      | 1.03 | 95.5              |
| Glucose + NaF           | 133.20        | 33.80        | 28.00       | 42.50       | 3.44        | 6.16                   | 39.60                | 76.00         |                         | 6.5      | .977 | 96.4              |
| Hexosediphosphate alone | 178.5         | 68.00        | 17.10       | 3.36        | 4.56        | 6.10                   | 24.95                | 90.70         |                         | 6.1      | 1.07 | 89.1              |

necessarily require the Embden-Meyerhof-Parnas mechanism to obtain their growth energy. Furthermore, the preference shown for glucose as a substrate when hexosediphosphate or phosphoglyceric acid is present, indicates that reactions involving these compounds may be of secondary importance in glycolysis by the living cell. It must not be overlooked, however, that the presence of these esters seems to exert a stimulative effect on the utilization of glucose, and it is quite possible that they may have a primary rôle in cell metabolism, even if they are not necessarily present as intermediates.

#### SUMMARY

The presence of phosphoglyceric acid and hexosediphosphate exerted a stimulative effect on the utilization of glucose by *A. indologenes*.

Sodium fluoride (0.02 M) diminished the rate of utilization of glucose and strongly inhibited the utilization of the phosphate esters in the presence of glucose.

In the absence of glucose, phosphoglyceric acid was not attacked appreciably, either in the absence or presence of sodium fluoride.

Glucose was fermented to normal final products in the presence of 0.02 M NaF by *A. indologenes*.

Hexosediphosphate was readily fermented to the same products as glucose.

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# THE GENERAL DIFFERENTIAL OPERATOR

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Received January 19, 1940

1. *Introduction.* The object of the present paper is to show how the differential equation

$$z \frac{\partial^2 V(x, z)}{\partial z^2} + (n + xz + 1) \frac{\partial V(x, z)}{\partial z} + n \times V(x, z) = 0$$

affords a novel basis for the unification of theories about general differentiation and integration. This equation also includes the formal theory of the logarithmic operator first introduced by V. Volterra in the study of integral equations with kernels of the form

$$K(x, t) = A \log^2(x-t) + B \log(x-t) + C.$$

The concept of a fractional operator was first used by Leibnitz, soon after the discovery of the calculus, who considered the meaning to be

attached to such operators as  $\frac{d^{\frac{1}{2}}y}{dx^{\frac{1}{2}}}$ . This idea was later developed, more

or less independently, by P. S. Laplace, N. H. Abel, J. Fourier, J. Liouville, G.F.B. Riemann, H. Laurent, O. Heaviside, and others. Unfortunately, there was not a unique definition of the operator, and the generalizations of Abel and Riemann were in apparent disagreement with those of Liouville. This discouraging situation led many to abandon the use of fractional operators, and A. De Morgan in his famous *Calculus* published in 1842 regarded the matter as being in confusion.

The ambiguities noted by De Morgan, however, were removed by the researches of Laurent,\* who approached the matter from the standpoint of the Cauchy formula

$$u^{(n)}(x) = \frac{n!}{2\pi i} \int_C \frac{u(t)}{(x-t)^{n+1}} dt.$$

If  $C$  is a closed path about  $x$  in the finite plane, if  $n$  is an integer, and if  $u(t)$  is an analytic function within the region enclosed by  $C$ , then we obtain the ordinary theory. But if  $n$  is a fraction, then the plane must be cut from  $x$  to infinity and  $C$  must be a path which does not cross this cut. Specialization of this path leads on the one hand to the definition of Liouville, and on the other to that of Abel-Riemann. It is the definition of the latter to which the results of this paper refer.

\* Laurent, H., Sur le calcul des dérivées à indices quelconques, *Nouvelles annales*, vol. 3, series 3, 1884, pp. 240-252.



2. *Definitions.* In order to attain a symbolic statement of the problem we shall introduce the operational symbol  $z^{-1}$ , which is defined by the following identity:

$$z^{-1} \rightarrow u(x) = \int_0^x u(t) dt$$

where the symbol  $\rightarrow$  means that the operator preceding the symbol operates upon the function following it.

If the function  $u(x)$  possesses a Taylor's expansion about the point  $t = x$ , then we may write  $u(t)$  symbolically in the form

$$u(t) = [1 + (t-x)z + \frac{(t-x)^2 z^2}{2!} + \dots] \rightarrow u(x) = e^{(t-x)z} \rightarrow u(x)$$

where  $z, z^2, z^3, \dots$ , are the first, second, third, ..., derivatives of  $u(x)$ .

Introducing this symbol into the preceding integral, we obtain

$$z^{-1} \rightarrow u(x) \equiv \int_0^x e^{(t-x)z} dt \rightarrow u(x) = \int_0^x e^{-sz} ds \rightarrow u(x).$$

It is obvious that the right hand symbol is equivalent to the left hand symbol when the function  $u(x)$  possesses a Taylor's expansion about the point  $t = x$ . The discussion is thus restricted to this class of functions, although some of the formal results which are obtained may be extended to a broader class of functions.

In general, we have

$$\begin{aligned} z^{-n} \rightarrow u(x) &= \int_0^x \dots \int_0^x u(t) dt^n = \int_0^x \frac{e^{(t-x)z} (x-t)^{n-1}}{(n-1)!} dt \rightarrow u(x) \\ &= \int_0^x \frac{e^{-sz} s^{n-1}}{(n-1)!} ds \rightarrow u(x), \end{aligned}$$

where the simple integral replaces the  $n$ -fold one by a well-known formula of integral equations.\*\* The operators

$$z^{-n} \text{ and } \int_0^x \frac{e^{-sz} s^{n-1}}{(n-1)!} ds$$

are called equivalent. The function defined by this integral will be designated by the symbol  $Q_n(x, z)$ , that is,

$$Q_n(x, z) = \int_0^x \frac{s^{n-1} e^{-sz}}{(n-1)!} ds.$$

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\*\* Michell and Belz, Mathematical analysis, 2: 586, formula (10).

3. *Derivation of the differential equation.* A first partial Z differentiation of the function  $Q_n(x, z)$  gives

$$\frac{\partial Q_n(x, z)}{\partial z} = - \int_0^x \frac{s^n e^{-sz}}{(n-1)!} ds.$$

and a second gives

$$\frac{\partial^2 Q_n(x, z)}{\partial z^2} = \int_0^x \frac{s^{n+1} e^{-sz}}{(n-1)!} ds.$$

From the last three equations we readily obtain

$$\begin{aligned} (1) \quad & z \frac{\partial^2 Q_n(x, z)}{\partial z^2} + (n + xz + 1) \frac{\partial Q_n(x, z)}{\partial z} + nx Q_n(x, z) \\ &= \int_0^x [zs^{n+1} - (n + xz + 1)s^n + nxs^{n-1}] \frac{e^{-sz}}{(n-1)!} ds. \end{aligned}$$

The integration of the first term of the integral by parts twice and the second term by parts once reduces the right hand member of the equation (1) to zero.

Hence we have a partial differential equation

$$(2) \quad z \frac{\partial^2 V_n(x, z)}{\partial z^2} + (n + xz + 1) \frac{\partial V_n(x, z)}{\partial z} + nx V_n(x, z) = 0$$

which has the function,  $Q_n(x, z)$ , as a solution. By substitution,  $z^{-n}$  is found to be a second solution.

The equation (2) has been derived under the assumption that the parameter  $n$  is an integer. However we shall discuss this question for all values of the parameter.

4. *Solutions of the equation.* It is easily shown that the functions

$$z^{-n} \text{ and } \int_0^x \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds$$

are formal solutions\*\*\* of equation (2) for all  $n$  except the negative integers.

\*\*\* These solution functions are the ordinary generalized integration operators defined by H. T. Davis in "A Survey for the Inversion of Integrals of Volterra Type" p. 32, Indiana University Studies, No. 76-77, and in "The Theory of the Volterra Integral Equation of Second Kind," Indiana University Studies Nos. 88-89-90.

In this case these solution functions reduce to  $z^n$  and

$${}_cD_x^{m+n} = \frac{\partial^{m+1}}{\partial x^{m+1}} \int_0^x \frac{(x-t)^{-n} e^{(t-x)z}}{\Gamma(-n)} dt. \quad \begin{matrix} (m=0, 1, 2, \dots), 0 < n < 1 \\ m=[n], \end{matrix}$$

and are identical with the generalized differentiation operator as defined by Davis in the articles previously cited.

We obtain another useful form of the operator  $Q_n(x, z)$ , from the equation (2) by replacing  $n$  by  $-a$ , and solving the resulting equation,

$$(3) \quad z \frac{\partial^2 u_a(x, z)}{\partial z^2} + (-a + xz + 1) \frac{\partial u_a(x, z)}{\partial z} - ax u_a(x, z) = 0 \quad a > 0$$

in series form.

The equation (3) becomes

$$(\theta^2 - (a - xz)\theta - axz) u_a(x, z) = 0$$

where  $\theta$  is  $z \frac{\partial}{\partial z}$ . The indicial equation

$$\varrho^2 - a\varrho = 0$$

has the roots 0 and  $a$ . Thus the series solutions of equation (3) are

$$u_1(x, z) = z^a; \quad u_2(x, z) = \frac{x^a}{\Gamma(-a)} \sum_{s=0}^{\infty} \frac{(-1)^s x^s z^s}{(a-s)s!}, \quad a \neq 1, 2, 3, \dots$$

according as  $\varrho = a$  or 0.

When  $a$  is a positive integer, the second solution must be modified. It is obtained by the usual method employed when the roots of the indicial equation differ by integers. The solution, namely,

$$(4) \quad u_2(x, z) = \sum_{s=0}^{\infty} (-1)^s \left[ \frac{a}{(a-s)s!} \left( \frac{s-2a}{a(s-a)} - \sum_{n=1}^{\infty} \frac{1}{n} + \log s \right) \right] x^s z^s$$

introduces the logarithmic operator.

5. *Logarithmic operators.* The logarithmic operator,  $z^{-n} \log z$ , appears first in the works of V. Volterra.<sup>††</sup> It satisfies the partial differential equation

$$(5) \quad z \frac{\partial^2 v_n(x, z)}{\partial z^2} + (n + xz + 1) \frac{\partial v_n(x, z)}{\partial z} + nx v_n(x, z) + \left(x - \frac{n}{z}\right) \frac{v_n(x, z)}{\log z} = 0$$

<sup>††</sup>Volterra, Vito, Leçons sur la composition et les fonctions permutables. (1924) Gauthier-Villars et C<sup>ie</sup>.

where

$$v_n(x, z) \equiv - \frac{\partial V_n(x, z)}{\partial n}$$

The differential equation (5) was obtained by differentiating each member of equation (2) partially with respect to  $n$  and making the preceding substitution.

Instead of obtaining the logarithmic operator from equation (5) it will be obtained in the following manner. The differentiation of each member of the operational identity

$$(6) \quad z^{-n} \equiv \int_0^x \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds$$

with respect to  $n$  gives

$$(7) \quad z^{-n} \log z \equiv \int_0^x [\Psi(n) - \log s] \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds$$

where  $\Psi(n)$  is the function  $\frac{\Gamma'(n)}{\Gamma(n)}$ .

Hence the logarithmic operator may be defined as

$$\lim_{n \rightarrow 0} z^{-n} \log z = \log z = \lim_{n \rightarrow 0} \int_0^x [\Psi(n) - \log s] \frac{s^{n-1} e^{-sz}}{\Gamma(n)} ds.$$

Example 1. Consider the operation  $\log z \rightarrow f(x)$ .

Starting with the relation (7), letting  $e^{-sz} \rightarrow f(x)$ , using the relation  $\Psi(n+1) - \Psi(n) = 1/n$ , integrating, and expressing in operational form, we obtain

$$(8) \quad z^{-n} \log z \rightarrow f(x) = (\Psi(n+1) - \log s) z^{-n} \rightarrow f(x) + \int_0^x \frac{z}{x} Q_{n+1}(x, z) dx \rightarrow f(x)$$

Letting  $n \rightarrow 0$  in each member of equation (8), we obtain

$$(9) \quad \log z \rightarrow f(x) = (-C - \log x) f(x) + \int_0^x \frac{z}{x} Q_1(x, z) dx \rightarrow f(x)$$

since  $\Psi(1) = -C$ , and where  $C$  is Euler's constant 0.577215-----.

If  $f(x) = 1$ , equation (9) becomes

$$\log Z \rightarrow 1 = -C - \log x,$$

which agrees with the result obtained by Davis.†††

††† Davis, H. T., Theory of linear operators, p. 78. Principia Press.



The operator inverse to  $z^{-n} \log z$  will be obtained formally by integrating  $z^{-\mu+n}$  with respect to  $\mu$  from zero to infinity. Thus

$$z^{n+1} \int_0^\infty z^{-(\mu+1)} d\mu = \frac{z^n}{\log z} = z^{n+1} \int_0^x \int_0^\infty \frac{s^\mu e^{-sz}}{\Gamma(\mu+1)} d\mu ds.$$

Example 2. Solve the integral equation

$$(10) \quad f(x) = \int_0^x (\log s + C) u(s) ds \quad (f(0) = 0).$$

In operational form the equation (10) is

$$f(x) = -z^{-1} \log z \rightarrow u(x),$$

and therefore

$$u(x) = -\frac{z}{\log z} \rightarrow f(x) = -z^2 \int_0^x \int_0^\infty \frac{s^\mu f(x-s)}{\Gamma(\mu+1)} d\mu ds.$$

From the foregoing analysis we see that a powerful instrument for the unification of theories about the fractional operator, its derivative symbol  $z^{-n} \log z$ , and other similar operators derived by various operations on the parameter  $n$ , is furnished by the differential equation (2). As Leibnitz once remarked about operational processes, there are doubtless other things hidden here.

# INSECTS AS VECTORS OF YELLOW DWARF, A VIRUS DISEASE OF ONIONS<sup>1</sup>

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Received January 10, 1940

In the spring of 1927 commercial onion growers in eastern Iowa became greatly alarmed over a malady which was causing serious losses to the onion crop. An investigation revealed the presence of an unknown disease. During the succeeding year (1928) the infection attained epiphytotic proportions, 30 to 40 per cent of the commercial plantings of the district being affected and in some fields more than 90 per cent of the plants became diseased. How and when it reached this territory is not definitely known. Information obtained from growers and others interested in onion production in this area indicates, however, that the disease probably had been present for a period of several years but unnoticed because of its relatively mild proportions.

The symptoms of the disease, the lack of any constantly associated organism, and a demonstration of its transmissibility indicated that the causal agent was a virus. The writer was employed by the Entomology and Economic Zoology Section of the Iowa Agricultural Experiment Station to study the entomological aspects of the problem, with reference to the part played by insects in the natural dissemination of the disease. These investigations were conducted during the period 1931 to 1935 under field conditions in the Pleasant Valley onion growing district and in the greenhouse and experimental fields at Ames.

## REVIEW OF LITERATURE

The disease was first described and referred to as "yellow dwarf" by Melhus et. al. (1928) and evidence of its virus nature was presented. That onion yellow dwarf can be transmitted by insects was first demonstrated by Drake, Harris, and Tate (1932). In succeeding publications (1933 and 1934) these workers presented evidence to show that the disease can be transmitted by a large number of different species of aphids

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<sup>1</sup> Journal Paper No. J-718 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 135.

<sup>2</sup> Condensed from a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Iowa State College, August, 1936.

<sup>3</sup> Grateful acknowledgement is made to Dr. C. J. Drake, under whose direction these studies were conducted, for valuable suggestions, advice and assistance; to Dr. H. M. Harris for valuable advice and assistance throughout the course of the investigations; to Messrs. R. M. Jones and L. H. Shropshire who conducted the initial phases of the project, and to other staff members of the Department of Zoology and Entomology, Iowa State College.

but they were unable to secure transmission with any other group of insects. A bulletin pertaining to the phytopathological phases of the disease in Iowa has been published by Henderson (1935).

In the "Plant Disease Reporter" for 1918 a mosaic of onions was reported as having been observed in West Virginia during the previous year (1917). During the succeeding year (1918) the disease caused a moderate amount of damage in two counties. In the same publication for 1929 it was stated that the disease is now considered as being identical with that occurring in Iowa.

According to Bremer (1929 and 1937) there is in Germany a disease of onions, referred to as "Gelbstreifigkeit", which is characterized by a "drooping, crinkling, and yellowing of the leaves associated with metabolic disturbances leading to a reduction of bulb and seed formation". This disease appears to be quite similar, if not identical, to that which occurs in Iowa.

Blattny (1930) has reported a disease of onions, referred to as onion yellows, in Central Europe (Czecho-Slovakia) which also appears to be similar to onion yellow dwarf. Henderson (1935) reported that specimens of yellow dwarf diseased plants had been collected both in Minnesota and California.

#### DESCRIPTION OF THE DISEASE

The initial symptoms of the disease, on plants grown from infected bulbs, are evident as chlorotic streaks or blotches only slightly lighter in color than the surrounding normal appearing green tissue and suggest a mosaic type of disease. As growth continues chlorosis becomes more pronounced and the spots elongated, thus producing a somewhat streaked appearance. Under some conditions, particularly in more nearly mature plants, a general chlorosis may develop in the affected parts. In advanced cases the plants are severely stunted, the leaves crinkled, flat and often drooped with the tips touching the ground.

Plants which become infected during the current season develop the first disease symptoms on the central or younger leaves 5 to 12 days after inoculation in the form of yellowish blotches or abbreviated streaks, which are irregular in outline, and give the affected leaves, in general appearance, a mild mosaic pattern. In advanced stages there is a more or less pronounced streaking and chlorosis may gradually become systemic in the above ground portions of the plant. The degree of stunting and amount of injury to the current growth varies considerably with the stage of growth of the plant at the time of infection, being more pronounced when infection occurs early.

On flower stems the disease first becomes apparent as more or less elongated streaks or blotches extending from the base upward. As the disease progresses the streaks coalesce, thus producing a general chlorotic appearance. In advanced stages the flower stem becomes twisted and curled and fails to produce a normal seed crop.

Plants which are fairly mature at the time of infection suffer little

damage during the current season and may or may not show any evidence of the disease but, if such bulbs are planted the following season, there will be a marked reduction in yield and the quality of an inferior grade.

Masking of symptoms is of common occurrence, particularly under unfavorable conditions of growth and in plants which are fairly mature at the time of infection.

#### PRELIMINARY STUDIES OF INSECT TRANSMISSION

Because of the similarity of yellow dwarf of onions to certain insect-borne diseases of other plants, such as sugar cane mosaic, aster yellow and others, it seemed logical to suspect that insects were responsible for its natural dissemination. A survey of the insect pests attacking onions in Iowa, including both primary and secondary forms, was made in an effort to determine which ones might be involved in spreading the disease. For many years the onion thrips, *Thrips tabaci* Lind. has been a more or less serious onion pest in Iowa. The tarnished plant bug, *Lygus pratensis* L. was found to be fairly abundant during certain seasons as were also several species of leaf hoppers, including *Cicadula sexnotata* Fall, *Deltocephalus inimicus* Say and *Empoasca fabae* Harris.

In addition, the bulb mite, *Rhizoglyphus hyacinthi* Boisd., the imported onion maggot, *Hylemyia antiqua* Meig., the black onion fly, *Tritoxa flexa* Wied., the seed corn maggot, *Hylemyia cilicrura* Rond., the barred wing onion fly, *Chaetopsis aenea* Wied., the lesser bulb flies, *Eumerus strigatus* Fall, and *E. tuberculatus* Fall and a number of other Dipterous maggots of secondary importance, were known to occur occasionally in considerable abundance.

In order to test the possibility of insect transmission a number of experiments were undertaken. In these tests either the Red Globe or Yellow Bottleneck varieties of onions, which were the most common commercial varieties grown in the Pleasant Valley district, were used as experimental plants.

#### EXPERIMENTS WITH THE ONION THRIPS, THRIPS TABACI LIND.

In view of the fact that the onion thrips, *T. tabaci*, was the most abundant and widely distributed insect pest of onions in Iowa, it was regarded with suspicion and it seemed advisable to investigate thoroughly the possibility of its serving as a vector of the yellow dwarf virus. For conducting the inoculation tests, which extended more or less interruptedly over a period of four years, various types of cages and procedures were employed.

In one series of tests an insect proof cage (4'x4'x6'), in which air circulation was obtained by means of an electric fan, was used to confine the thrips. Nymphs of *T. tabaci* were transferred to diseased onion plants within the cage and these served as the initial stock supply. After these had increased to the point of a fairly heavy infestation by breeding on diseased onion, healthy sets, from which all insects had been removed



by sterilization methods, were planted in the cage. These plants were left in the infection cage for a period of approximately 7 days, after new growth had started, and then removed to a more favorable growing environment in an "insect free" room of the greenhouse and examined daily over a period of 40 to 50 days for the development of yellow dwarf symptoms. These tests which included 212 plants resulted in no successful virus transmissions.

Similar experiments were conducted in which diseased onion plants growing in the cages were permitted to become infected with thrips and, after a heavy population had developed, healthy sets were planted in the cages alongside the thrips infested diseased plants and there grown to maturity. By this method approximately 500 healthy plants were exposed but no transmissions occurred.

The bulbs of 200 plants which were exposed to thrips in the above experiments and then grown to maturity were planted in the greenhouse the following season to determine if disease symptoms might develop during the second growth period. All of these remained healthy.

During the winter months (Feb., March, and April) of 1932 an experiment was conducted in which an "insect proof" compartment of the greenhouse was used as the experimental cage. Diseased bulbs were planted at weekly intervals in the soil benches and allowed to become heavily infested with onion thrips, which had been reared for two or more generations on diseased onions. Approximately 2500 healthy bulbs were then planted among the diseased plants so that there would be an opportunity for the thrips to migrate in a normal manner from diseased to healthy plants. The healthy plants soon became heavily infested with thrips but no yellow dwarf symptoms developed during the 3 months that they were kept under observation. Approximately 500 of these bulbs were regrown the following fall but no evidence of transmission was obtained. Duplicate series of experiments, including more than 1000 plants, about half of which were carried through a second growth period, were later conducted but with negative results.

A number of other types of "insect proof" cages which it is not thought necessary to describe at this time were employed. A total of more than 3500 plants were exposed to nymphs and adults of *T. tabaci* which had fed for 2 or more successive generations on diseased onion but no successful transmissions were obtained.

These negative results together with confirmatory field evidence indicate that *T. tabaci* is unable to transmit the virus of onion yellow dwarf from diseased to healthy plants.

#### EXPERIMENTS WITH LEAFHOPPERS

Although there were no species of leafhoppers which were known normally to breed on the onion plant or feed on it to any appreciable extent in Iowa, field observations showed that certain species such as *Cicadula sexnotata* (Fall.), *Deltoccephalus inimicus* (Say), *Empoasca*

*fabae* (Harris) and others often could be found in onion fields in considerable numbers.

In June 1931 a series of experiments were conducted with *C. sexnotata*. Approximately one-fourth of an acre of land located about 2 miles from the infected area was obtained for use in transmission studies. Set onions produced in disease free areas were planted at frequent intervals to insure a constant supply of susceptible host plants in the most desirable stage of growth. Large numbers of leafhoppers were collected 3 to 5 times each week for several weeks, either in onion fields or nearby in the infected area, and confined on diseased onion plants for varying intervals of time ranging from 1 to 4 days.

In some of the experiments the leafhoppers were removed from the diseased plants and caged directly on healthy ones in the experimental plots. In others, they were caged on their normal host plants, after being confined on diseased plants, for varying periods of time up to 11 days and then transferred to healthy onions. The cages usually remained over the experimental plants until all insects had perished, which was ordinarily 4 or 5 days, but in case any were still alive at this time they were destroyed either by hand or by means of sprays in order to prevent chance inoculation. A total of 340 plants were exposed to *C. sexnotata* in this manner. None were visibly infected at the time of harvesting the bulbs during the early part of July (1931).

Parallel experiments involving 205 plants were conducted with *D. inimicus* but, likewise, the results were negative.

All of the plants exposed to the two species of leafhoppers mentioned above were brought to Ames and regrown during the following December (1931) in the greenhouse in order to determine if yellow dwarf symptoms might develop during the second growth period. All results were negative.

Somewhat similar experiments were conducted with *C. sexnotata* during July, August, and September, 1931 in the experimental plots at Ames. Altogether 212 healthy onion plants were exposed to leafhoppers, which had previously fed on diseased onion, with negative results. These plants also were harvested and regrown in the greenhouse during the following winter months but none of them developed yellow dwarf symptoms.

During the fall and winter of 1931-32 the leafhopper *C. sexnotata* was colonized on asters in "insect proof" cages in the greenhouse at Ames for use in transmission experiments. The leafhoppers were confined on diseased onion plants in large lantern globe cages for varying periods of time, depending upon the individual experiment in progress. In some cases they were transferred directly from diseased to healthy onion while in others they were confined on asters for a given interval of time before being transferred to healthy onion plants. In some instances the insects were transferred from diseased to a succession of healthy onion plants. The idea involved, in the various methods of handling the insects, was to

take advantage of the fact that there might be a period of delayed infectivity in the leafhoppers such as has been shown to occur by Kunkel (1926) in the development of aster yellows and *C. sexnotata*. Through the procedure herein described 512 healthy onion plants were exposed but, with one exception, all remained healthy during the period which they were kept under observation. The one plant which became diseased in this series seems to be the result of an accidental transmission, since numerous attempts to obtain additional transmissions were unsuccessful.

During June and July 1931 potato leafhoppers, *E. fabae*, were confined on diseased onions for an interval of one or two days and then transferred to healthy onion plants. During the period of observation the cages remained over the experimental plants until all insects had died, which was usually from 3 to 5 days. In some cases the transfers were made from diseased onion back to potato plants for an interval of time before exposure of healthy onion plants. Of 147 healthy plants exposed, none developed yellow dwarf symptoms during the current season or when they were regrown the following season in the greenhouse.

From these experiments it was concluded that neither *C. sexnotata*, *D. inimicus* nor *E. fabae* were capable of transmitting the virus of onion yellow dwarf from diseased to healthy plants.

#### EXPERIMENTS WITH THE TARNISHED PLANT BUG, *LYGUS PRATENSIS* L.

On onion plants the injury caused by the feeding of the tarnished plant bug, *L. pratensis*, first becomes apparent as a small "water-soaked" area which gradually turns brown and may develop into a necrotic spot. In the onion growing district at Pleasant Valley such injury has been observed to result in somewhat serious damage in local areas. These bugs quite commonly congregate in large number in the flowers of onion and their feeding activities may cause a considerable reduction in yield of seed.

During the spring and fall of 1931 and 1932 a series of experiments were conducted in which tarnished plant bugs were confined on diseased onion for 24- to 48-hour periods, and then transferred to healthy plants. In these experiments it was found desirable to use large cages (1'x1'x2') covered with cheese cloth which served as a partial shade. If more than 6 or 8 individuals were confined on a small onion plant for a few hours their feeding activities frequently produced such severe effects as to either kill the plant completely or cause a marked stunting. This being the case, the experimental plants were usually caged with not more than 3 or 4 bugs which were removed after a 12- to 24-hour period.

A total of 94 healthy onion plants were caged with the tarnished plant bug with negative results. Most of these plants were regrown, but no evidence of the disease appeared.

#### EXPERIMENTS WITH ONION MIRIDS, *LABOPIDIA AINSLEI* KNIGHT AND *L. ALLI* KNIGHT

Two species of plant bugs, *L. ainslei* and *L. alli*, which normally feed and breed on onions, both wild and cultivated, and a few closely related



plants such as garlic, occur quite commonly in Iowa. Although a detailed biological study of these 2 species of insects has not been made, it has been determined that only one generation of each species is produced annually. The active stages are present for only a short time in early spring, being present the remainder of the year as eggs which are deposited in the host plant tissues. During the springs of 1930 to 1933, inclusive, 468 nymphs and adults, collected in the field, were caged on diseased onion for a period of 2 days and then transferred to large "insect proof" cages containing young healthy onion plants. All of these, which included 174 plants, remained healthy during the current growth period and when regrown the following season.

#### EXPERIMENTS WITH THE BULB MITE, RHIZOGLYPHUS HYACINTHI BOISD.

Under favorable conditions of temperature and humidity the bulb mite, *R. hyacinthi*, constitutes a pest of serious consequence to the onion crop, especially to stored bulbs and bulbs which are allowed to remain in the field sometime after becoming mature. Since it is a pest so intimately associated with the onion crop, the possibility of some relationship between it and the spread of yellow dwarf seemed tenable.

During the spring, summer, and fall months of 1930-31 a series of transmission experiments with the bulb mite were conducted. Inoculated bulb mites, obtained both on diseased plants which had become infested under natural conditions and on plants which had been experimentally infested, were transferred along with the diseased bulbs to healthy plants in "insect proof" cages. The plants remained caged for a period of 2 to 3 weeks. Of approximately 200 healthy plants exposed in this manner, none developed yellow dwarf. About half of these plants were kept under observation during a second growth period but all remained healthy.

#### EXPERIMENTS WITH BULB INFESTING MAGGOTS

The imported onion maggot, *Hylemyia antiqua* Meig., and the seed corn maggot, *Hylemyia cilicrura* Rond., although pests which have not occurred in serious proportions up to the present time in Iowa, occasionally may attain such numbers as to destroy 5 to 15 per cent or more of the onion crop in local plantings. In general, such a condition prevailed during the period (1928-1931) when yellow dwarf was widespread in the Pleasant Valley onion growing district.

In spite of the fact that insects of this type appear to be poorly adapted for transmitting a virus disease and are not known to have been implicated previously in any instance, the importance and close relationship of these three species of dipterous maggots to the onion crop suggested a possible relationship.

For confining the maggots on healthy plants, large glass cages (1'x2½'x4') were used. These cages were placed over a group of plants which were growing in a greenhouse bench, and inoculated maggots, obtained from either naturally or experimentally infested diseased bulbs, introduced. After a period of about 2 weeks the cages were removed and



the plants kept under observation until time of harvest. As was expected, a few of the experimental plants were killed as a result of the maggot attacks and others were severely stunted. More than 200 plants were exposed in this manner with negative results. A considerable number of these plants were regrown but no yellow dwarf symptoms developed.

During the spring of 1931 a series of experiments were conducted in which inoculated maggots, obtained from naturally infested diseased bulbs, were introduced into small incisions made in the bulb of young healthy growing onion plants. Of approximately 250 plants exposed by this method, none became diseased.

#### EXPERIMENTS WITH MEALY BUGS

While conducting transmission experiments with the various groups of sucking insects, it was thought advisable to include certain species of mealy bugs which commonly occur on greenhouse plants and are practically always available in large numbers.

In March 1933 mealy bugs (*Phenacoccus* sp.) were collected in the greenhouse at Ames and confined on diseased onion plants, an effort being made to select the younger and more active stages. After 24 hours they were transferred to healthy onion plants. While confined on the plants it was noted that many individuals did not appear to be feeding and considerable numbers were congregated either on the side of the cage or on the ground surface.

Twenty-eight healthy plants were exposed in this series of experiments all of which remained healthy, with one exception. Additional experiments were later conducted in an effort to verify the previous transmission but without success. It was concluded, therefore, that the one diseased plant either was the result of an accidental infection or that yellow dwarf transmission by mealy bugs is an extremely rare occurrence.

Experiments similar to the ones just described with the mealy bug, *Pseudococcus citri* Risso, collected from coleus plants, also gave negative results.

#### EXPERIMENTS WITH APHIDS

Previous to the outbreak of yellow dwarf in Iowa there was little occasion for giving serious consideration to insects which only occasionally occur on onion. Following the advent of yellow dwarf, however, and the failure to incriminate any of the primary and secondary onion pests as vectors of the disease, attention was directed toward insects which only occasionally or incidentally feed upon the virus host plant. Since aphids comprise the most important group of plant virus vectors, at least from the standpoint of number of diseases transmitted, attention quite naturally was directed toward this group as possible vectors.

Field observations made during the period from 1928-1931 showed that often many different species of plant lice were present in onion fields either breeding on weed hosts or as migrants and that these insects fed

on growing onion plants to some extent. A detailed discussion of this subject will be given in a subsequent section of this paper.

In December, 1931, several hundred individuals of the bean aphid, *Aphis rumicis* L., which were being colonized on nasturtium, were confined on a diseased onion plant for a period of two days and then transferred to young healthy onion plants. In this experiment 8 plants were used and at the end of approximately three weeks five plants had developed typical yellow dwarf symptoms. Additional experiments were subsequently conducted with *A. rumicis* and with a number of other species of aphids. A detailed account of these tests is given in the following discussion.

## TRANSMISSION EXPERIMENTS WITH APHIDS

### MATERIALS AND METHODS

A stock supply of plant lice was maintained by colonization on one or more of their normal host plants grown either in small "insect proof" cages or in isolated compartments of the greenhouse. Transfers were made either with a small camel-hair brush or an aspirator similar to the type described by Kunkel (1926) for use in handling leafhoppers. For confining aphids on diseased plants large lantern globe cages were employed. During the early part of the work cylindrical glass tubes approximately 2.5 inches in diameter and 12 inches long and covered over with cheese cloth, held in position by means of a rubber band, were used to confine the insects on individual experimental plants. Since there is a tendency for moisture to condense on the sides of small glass cages, particularly when exposed to sunlight, celluloid and cellophane cages of a similar size and shape were substituted and these proved to be somewhat more satisfactory.

The aphids were confined on a diseased plant for a given period of time and then transferred directly to the experimental plant, as a general rule at the rate of 30 to 50 individuals per plant. Except for certain types of experiments, which will be discussed later in the text, the insects were kept on the experimental plant for a period of 2 or 3 days following which the cages were removed and the plants carefully sprayed with either a solution of pyrethrum or nicotine sulphate and soap. All experimental plants were accompanied by an equal or greater number of controls.

Experimental plants were grown from sets, three-eighths to one-half inch in size, which were produced in disease free areas and no cases of natural infection were known to have occurred. As a rule the plants were used when one to four inches in height, more satisfactory results being obtained with plants of this age than with older or more mature plants.

All plants in the experimental greenhouse were treated at frequent intervals with either a nicotine sulphate-soap or pyrethrum spray. Occasionally the sprays were supplemented with fumigation by means of vaporizing free nicotine as an additional precaution against infestations of extraneous insects.

## TRANSMISSION BY APHIS RUMICIS L.

Because of the abundance, wide distribution and convenience with which it could be reared in the laboratory, *A. rumicis* was selected as the most satisfactory species of aphid to use in large scale transmission studies. Vigorous colonies were maintained on dwarf nasturtium grown in board flats (1'x2'x6"). In order to have as nearly a homogenous population as possible, the stock supply of aphids was developed originally from a single parthenogenetic female.

In transmission experiments conducted during the year 1931-1932, 332 healthy onion plants were exposed to viruliferous *A. rumicis*. Of these 236 or an average of 71.0 per cent developed mosaic. The initial symptoms of the disease developed in a relatively short time, being 7 to 12 days in most cases with an average of 10.6. The range, however, was from 4 to 29 days. The incubation period of the disease was markedly influenced by the age and state of growth of the plant and by the greenhouse temperature, being comparatively longer in older plants in a poor state of growth and when the temperature was sufficiently high to retard their growth.

RAPIDITY WITH WHICH *A. RUMICIS* BECOMES INFECTIVE

A series of experiments was conducted to determine the period of time necessary for *A. rumicis* to feed on a diseased plant in order to become infective. With insects which do not normally feed on the virus host plant and apparently accept such plants as a source of food only during their wanderings or migrations, or in case of necessity because of the absence of their normal host plant, this point has considerable influence on the effectiveness with which the vector may disseminate the virus. In order to establish this relationship, colonies of *A. rumicis* were confined on diseased onion for varying periods of time and then transferred to healthy onion plants. In preliminary experiments it was determined that the period of time was relatively short—less than 48 hours. With this range established, aphids were confined on diseased plants for periods of time varying from 30 minutes to 48 hours. The results of these tests are presented in table 1. It will be noted that there does not appear to be any significant difference in the percentage of transmission obtained within this range.

In one instance successful transmission was obtained by a single aphid which had been confined on a diseased plant for only 15 minutes. These tests established the fact that extremely short feeding periods on a diseased plant are sufficient for the aphid vector to become infective.

## INCUBATION OF THE VIRUS IN APHIS RUMICIS

Preliminary experiments indicated that if there was a period of delayed infectivity in the insect it was less than 60 hours. Colonies of aphids were confined on diseased plants for periods of 3 to 12 hours and then transferred to a succession of healthy onion plants. Approximately 50 aphids, consisting principally of adult apterous forms were confined



TABLE 1. Infections obtained with *Aphis rumicis* when confined on diseased plants for varying periods of time

| Period of time<br>on diseased plants<br>(in hrs.) | Number<br>healthy plants<br>exposed | Number<br>plants<br>infected | Percentage<br>transmission |
|---|-------------------------------------|------------------------------|----------------------------|
| 48  | 64                                  | 48                           | 75.0                       |
| 24  | 53                                  | 40                           | 75.4                       |
| 12  | 32                                  | 21                           | 65.6                       |
| 5   | 16                                  | 9                            | 56.2                       |
| .5  | 34                                  | 22                           | 64.7                       |

on each healthy plant. The use of large numbers of aphids was desirable since all the insects placed on diseased plants, do not become infective and, when transferred to healthy plants, all individuals do not feed, especially during short exposures. Moreover, during the process of transferring aphids, many of them become disturbed to such an extent that considerable time may be spent in wandering about in the cage in an effort to escape. The results of these tests are shown in table 2.

Five experiments were conducted each of which consisted of six colonies of aphids. By referring to table 2 it will be noted that, with one exception, all the successful transfers were obtained during exposures in which the aphids had not been removed from the source of inoculum for more than three to four hours at the time of making transfers. In Experiment 3 a single transmission was obtained after the aphids had been removed from the diseased plant for a period of 21.5 hours. These data indicate that there is no incubation period of the virus in *A. rumicis*, or at most an extremely short one, and that infective power is rapidly lost when aphids are confined on healthy susceptible hosts.

#### RETENTION OF INFECTIVE POWER BY APHIDS

From the data presented in table 2 it is quite evident that in the process of transferring viruliferous *A. rumicis* to a succession of healthy susceptible plants the infective power is rapidly diminished, and completely lost after a few hours. Two other procedures were followed in an effort to establish a further understanding of this relationship. By the first method, aphids were confined on diseased plants and then transferred for a period of time to immune plants, following which they were transferred back to healthy susceptible plants. By the second method, aphids were caged on diseased plants and then confined without food in constant temperature chambers for varying periods of time.

#### *Retention of infective power when confined on immune plants*

Three species of aphids were used in this experiment, namely, the bean aphid, (*A. rumicis*) the cabbage aphid, (*Brevicoryne brassicae* L.), and the corn leaf aphid, (*Aphis maidis* Fitch), the normal immune hosts



TABLE 2. Infections obtained with 6 colonies of *Aphis rumicis* when confined on diseased onion plants for 3 to 12 hours and then transferred to a succession of healthy plants

| Experiment 1 |                    |          |          |          |          |          |          |
|--------------|--------------------|----------|----------|----------|----------|----------|----------|
| Period       | Length of exposure | Colony 1 | Colony 2 | Colony 3 | Colony 4 | Colony 5 | Colony 6 |
| 1            | 16                 | ×        | —        | —        | —        | —        | —        |
| 2            | 24                 | —        | —        | —        | —        | —        | —        |
| 3            | 24                 | —        | —        | —        | —        | —        | —        |
| Experiment 2 |                    |          |          |          |          |          |          |
| 1            | $\frac{1}{2}$      | —        | —        | —        | ×        | —        | —        |
| 2            | $2\frac{1}{2}$     | ×        | —        | ×        | ×        | ×        | —        |
| 3            | 18                 | ×        | —        | —        | —        | —        | —        |
| 4            | 45                 | —        | —        | —        | —        | —        | —        |
| Experiment 3 |                    |          |          |          |          |          |          |
| 1            | $\frac{1}{2}$      | ×        | —        | ×        | ×        | —        | —        |
| 2            | 16                 | —        | ×        | ×        | ×        | ×        | ×        |
| 3            | 5                  | ×        | —        | —        | —        | —        | —        |
| 4            | 3                  | —        | —        | ×        | —        | —        | —        |
| 5            | 16                 | —        | —        | —        | —        | —        | —        |
| Experiment 4 |                    |          |          |          |          |          |          |
| 1            | 24                 | ×        | ×        | —        | —        | —        | —        |
| 2            | 24                 | —        | —        | —        | —        | —        | —        |
| 3            | 24                 | —        | —        | —        | —        | —        | —        |
| Experiment 5 |                    |          |          |          |          |          |          |
| 1            | $\frac{1}{2}$      | ×        | —        | ×        | ×        | ×        | —        |
| 2            | 2                  | —        | —        | —        | —        | —        | —        |
| 3            | 24                 | —        | —        | —        | —        | —        | —        |
| 4            | 24                 | —        | —        | —        | —        | —        | —        |
| 5            | 24                 | —        | —        | —        | —        | —        | —        |

employed being, nasturtium, young cabbage plants and corn plants, respectively. The results of these tests appear in table 3.

After being confined on diseased onion plants, colonies of *A. rumicis* were transferred to nasturtium for periods of time ranging from 2 hours to 139 hours and then transferred to susceptible plants (onion). By reference to table 3, it will be noted that in no case were successful transmissions obtained after the aphids had been confined on immune plants for more than 36 hours.

In the case of cabbage aphids *B. brassicae*, 24 of 48 healthy plants to which direct transfers were made developed mosaic while of 16 plants exposed to aphids which had fed on immune plants for an interval of 24 hours, after removal from diseased plants, none became diseased.

Somewhat similar results were obtained with the corn leaf aphid. Of 16 healthy onion plants which were exposed immediately after removal of insects from diseased plant, 10 became diseased, while of 18 healthy onion plants exposed to aphids which had fed on corn plants for an interval of 24 hours or more after removal from diseased plants, none developed mosaic.

TABLE 3. Infections obtained with 3 species of aphids when confined for 24 hours on diseased plants and then transferred to plants immune to yellow dwarf for varying intervals of time before being caged on healthy onion plants

| Hours confined<br>on immune<br>plant | Number<br>healthy plants<br>exposed | Number<br>plants<br>infected | Percentage<br>transmission |
|--------------------------------------|-------------------------------------|------------------------------|----------------------------|
| <i>Aphis rumicis</i>                 |                                     |                              |                            |
| 2                                    | 12                                  | 2                            | 16.0                       |
| 12                                   | 6                                   | 0                            | 0.0                        |
| 17                                   | 18                                  | 2                            | 11.1                       |
| 24                                   | 24                                  | 0                            | 0.0                        |
| 36                                   | 6                                   | 1                            | 16.6                       |
| 43-139                               | 72                                  | 0                            | 0.0                        |
| <i>Brevocornye brassicae</i>         |                                     |                              |                            |
| 0                                    | 48                                  | 24                           | 50.0                       |
| 24                                   | 16                                  | 0                            | 0.0                        |
| <i>Aphis maidis</i>                  |                                     |                              |                            |
| 0                                    | 16                                  | 10                           | 62.5                       |
| 24                                   | 6                                   | 0                            | 0.0                        |
| 48                                   | 6                                   | 0                            | 0.0                        |
| 72                                   | 6                                   | 0                            | 0.0                        |

*Retention of infective power by A. rumicis when confined without food*

The question of how long viruliferous aphids retain their infective power when confined without food has a rather important bearing on their capacity to disseminate the yellow dwarf virus under field conditions. Under the influence of the migratory impulse or any other condition that may serve to initiate movement, plant lice in some instances undoubtedly subsist for considerable periods during which they take

little or no food. In determining how long *A. rumicis* retains its infective power under such conditions, colonies of aphids were confined on diseased plants for 6-hour periods and then transferred to a constant temperature chamber (70°F. and 75 per cent relative humidity) for varying periods of time ranging from 3 to 63 hours. The results of these tests appear in table 4. It will be noted that no successful transmissions were obtained after the aphids had been confined for more than 8 hours without food. Although a considerable number of individuals died within the first 24 to 48 hours many of them remained active for more than 2.5 days.

From this and the two preceding experiments it is apparent that plant lice retain their infective power for only a few hours after removal from diseased plants regardless of whether they are feeding on healthy susceptible hosts, immune hosts, or confined without food.

TABLE 4. Infections obtained with *Aphis rumicis* when fed on diseased plants for 6 hours and then confined without food for varying intervals of time before being transferred to healthy onion plants

| Number hours confined without food | Number healthy plants exposed | Number plants infected |
|------------------------------------|-------------------------------|------------------------|
| 3                                  | 5                             | 1                      |
| 4                                  | 5                             | 3                      |
| 5                                  | 11                            | 2                      |
| 6                                  | 5                             | 0                      |
| 7                                  | 5                             | 0                      |
| 8                                  | 11                            | 2                      |
| 9                                  | 10                            | 0                      |
| 10                                 | 5                             | 0                      |
| 12-63                              | 192                           | 0                      |

#### TRANSMISSION EXPERIMENTS WITH VARIOUS SPECIES OF APHIDS<sup>4</sup>

During the course of both field observations and experimental work on insects as related to the transmission of yellow dwarf of onion, many different species of aphids were taken on the foliage of onions, weeds in onion fields, and cultivated and wild plants in bordering and nearby areas. In preliminary experiments it was found that several species of plant lice, in addition to the one originally involved, namely, the bean aphid, *A. rumicis*, were capable of transmitting the virus of yellow dwarf from diseased to healthy plants. It was of considerable interest, therefore, to determine whether or not any species of aphids that could be

<sup>4</sup> Credit is due Dr. Floyd Andre, formerly of the Iowa Agricultural Experiment Station, for the greater part of the field and laboratory work involved in conducting and interpreting the results of this series of experiments. Credit is also due Dr. F. C. Hottes for the identification of a number of the species of aphids used and for verification of the author's determination of a number of other species.

TABLE 5. Infections obtained with 51 species of aphids when confined on diseased onion for a period of 12 to 48 hours and then transferred to healthy onion plants

| Species of aphid                               | Number healthy plants exposed | Number of infected plants* | Percentage transmissions |
|--|-------------------------------|----------------------------|--------------------------|
| <i>Amphorophora rossi</i> H. & F. ....         | 12                            | 5                          | 41.7                     |
| <i>Aphis ageratoidis</i> Oestlund .....        | 8                             | 5                          | 62.5                     |
| <i>A. cardui</i> Linn. ....                    | 4                             | 2                          | 50.0                     |
| <i>A. decepta</i> H. & F. ....                 | 10                            | 8                          | 80.0                     |
| <i>A. forbesi</i> Weed .....                   | 9                             | 5                          | 55.5                     |
| <i>A. gossypii</i> Glover .....                | 29                            | 24                         | 82.7                     |
| <i>A. helianthi</i> Monell .....               | 18                            | 14                         | 77.7                     |
| <i>A. laburni</i> Kalt. ....                   | 12                            | 9                          | 75.0                     |
| <i>A. maidis</i> Fitch .....                   | 94                            | 73                         | 77.6                     |
| <i>A. oenotherae</i> Oestlund.....             | 6                             | 2                          | 33.3                     |
| <i>A. oestlundii</i> Gillette .....            | 14                            | 10                         | 71.4                     |
| <i>A. pomi</i> DeGeer .....                    | 26                            | 23                         | 88.4                     |
| <i>A. rubi</i> Kaltenbach .....                | 5                             | 2                          | 40.0                     |
| <i>A. rumicis</i> Linn .....                   | 332                           | 236                        | 71.0                     |
| <i>A. sambucifoliae</i> Fitch .....            | 12                            | 3                          | 25.0                     |
| <i>A. vibrunicola</i> Gillette .....           | 7                             | 4                          | 57.1                     |
| <i>Brevicoryne brassicae</i> (Linn.) .....     | 39                            | 20                         | 51.2                     |
| <i>Calaphis betulella</i> Walsh .....          | 13                            | 8                          | 61.5                     |
| <i>Capitophorus flaveolus</i> (Walker) .....   | 15                            | 13                         | 86.6                     |
| <i>C. ribis</i> (Linn.) .....                  | 5                             | 4                          | 80.0                     |
| <i>Chaetophorus quercicola</i> (Monell) .....  | 14                            | 7                          | 50.0                     |
| <i>C. viminalis</i> Monell .....               | 8                             | 4                          | 50.0                     |
| <i>Cinara pini</i> Linn. ....                  | 12                            | 0                          | 0.0                      |
| <i>Drepanaphis acerifoliae</i> (Thomas) .....  | 20                            | 11                         | 55.5                     |
| <i>Eulachnus rileyi</i> Williams .....         | 9                             | 0                          | 0.0                      |
| <i>Hyalopterus atriplicis</i> (Linn.) .....    | 48                            | 30                         | 62.5                     |
| <i>H. pruni</i> (Geoffrey) .....               | 14                            | 9                          | 64.2                     |
| <i>Hysteroneura setariae</i> (Thomas) .....    | 14                            | 10                         | 71.4                     |
| <i>Macrosiphum ambrosiae</i> (Thomas) .....    | 25                            | 24                         | 92.3                     |
| <i>M. gei</i> (Koch) .....                     | 35                            | 16                         | 45.7                     |
| <i>M. gravicornis</i> Patch .....              | 17                            | 15                         | 88.2                     |
| <i>M. impatiensicolens</i> Patch .....         | 10                            | 9                          | 90.0                     |
| <i>M. pisi</i> (Kalt.) .....                   | 80                            | 65                         | 81.2                     |
| <i>M. purpurascens</i> (Oestlund) .....        | 5                             | 2                          | 40.0                     |
| <i>M. rosae</i> (Linn.) .....                  | 16                            | 16                         | 100.0                    |
| <i>M. rudbeckiae</i> (Fitch) .....             | 20                            | 16                         | 80.0                     |
| <i>Microsiphum artemisiae</i> (Gillette) ..... | 7                             | 5                          | 71.3                     |
| <i>Monellia caryae</i> (Monell) .....          | 10                            | 8                          | 80.0                     |
| <i>M. caryella</i> (Fitch) .....               | 10                            | 7                          | 70.0                     |
| <i>Myzocallis alhambri</i> Davidson .....      | 10                            | 6                          | 60.0                     |
| <i>M. asclepiadis</i> (Monell) .....           | 16                            | 12                         | 73.0                     |
| <i>M. ononidis</i> (Kaltenbach) .....          | 14                            | 9                          | 64.2                     |
| <i>Myzus cerasi</i> (Fabricius) .....          | 7                             | 2                          | 28.5                     |
| <i>Myzus persicae</i> (Sulzer) .....           | 48                            | 30                         | 62.5                     |
| <i>Periphyllus negundinis</i> (Thomas) .....   | 4                             | 1                          | 25.0                     |
| <i>Prociphilus fraxinifolii</i> (Riley).....   | 6                             | 0                          | 0.0                      |
| <i>Rhopalosiphum nymphaeae</i> (Linn.) .....   | 23                            | 19                         | 82.6                     |
| <i>R. prunifoliae</i> (Fitch) .....            | 121                           | 87                         | 71.9                     |
| <i>R. pseudobrassicae</i> (Davis) .....        | 10                            | 8                          | 80.0                     |
| <i>R. rhois</i> Monell .....                   | 16                            | 10                         | 62.5                     |
| <i>Thripsaphis balli</i> (Gillette) .....      | 10                            | 9                          | 90.0                     |

\* In all cases the number of controls was equal to or greater than the number of experimental plants. None of the controls developed yellow dwarf.



induced to feed on onion plants was capable of transmitting the virus. As a result, various species of aphids were collected in the vicinity of Ames and Pleasant Valley, Iowa, confined on diseased onion for a period of 12 to 24 hours, and then transferred to young healthy onion plants. The results of these tests appear in table 5.

It will be noted in table 5 that of 51 species tested, 48 are recorded as having transmitted the disease, a number which is sufficiently large to indicate that any species of aphid is potentially a vector of onion yellow dwarf.

As would be expected, all species of aphids and particularly those which normally feed principally on trees and shrubs, do not readily feed on onion plants. When large numbers were confined on onion plants, however, no difficulty was experienced in most cases in securing a reasonably high per cent of successful transmissions. Contrary to what might be suspected, a number of the non-migratory species, such as *M. alhambri*, which normally feed throughout the year on oak trees, *Quercus* spp., and *M. asclepiadis*, which is normally confined to milkweed species (*Asclepias* spp.), were collected in onion fields in large numbers during certain periods as alatae.

#### MASKED SYMPTOMS; VISIBLY UNINFECTED PLANTS AS A SOURCE OF INFECTION

Early in the work with onion yellow dwarf it was found that many plants which became inoculated during the current growth period failed to show visible infection until the bulbs were regrown the following season. Such a condition may be the result of a number of influences the more important of which are perhaps the stage of growth of the plant and its growing condition at the time infection occurs.

A series of experiments were conducted to determine if such plants might serve as a source of inoculum for aphid vectors. On seven plants which had been exposed to infective aphids but had failed to develop visible yellow dwarf symptoms, colonies of *A. rumicis* were confined for 24-hour periods and then transferred to young healthy onion plants. The results of these tests are shown in table 6. It will be noted that of 34 healthy plants exposed, only 7 developed visible symptoms and that these infections were obtained from 2 of the original 7 plants that were being used as a source of inoculum. These experiments, although of a limited scope, are sufficient to establish the fact that masking of symptoms does occur and that aphids feeding upon such plants may become infectious and spread the disease to healthy plants.

#### FIELD STUDIES OF APHID POPULATIONS

During 1932 a field station was maintained in the onion growing district at Pleasant Valley, Iowa and daily observations made in representative fields throughout the growing season for the purpose of determining the trend of aphid populations. Observations, also, were made

TABLE 6. Infections obtained with *Aphis rumicis* when confined on plants which previously had been exposed to viruliferous aphids, but failed to develop visible yellow dwarf symptoms, and then transferred to healthy onion plants

| No. of plants used in test | No. healthy plants exposed | No. plants infected |
|----------------------------|----------------------------|---------------------|
| 1                          | 5                          | 0                   |
| 1                          | 3                          | 0                   |
| 1                          | 4                          | 3                   |
| 1                          | 7                          | 0                   |
| 1                          | 4                          | 0                   |
| 1                          | 5                          | 0                   |
| 1                          | 6                          | 4                   |
| TOTAL 7                    | 34                         | 7                   |

at various times during the years 1930 and 1931 and from 1933 to 1935, inclusive.

The first aphids to appear in the onion fields in noticeable numbers were pea aphids, *M. pisi*. They were observed feeding on onion plants on May 10. On May 13, in fields located near alfalfa, an estimate based on counts made at various points showed that *M. pisi* was present in onion fields at the rate of about 2500 per acre. Alfalfa fields in the area under observation became extremely heavily populated with *M. pisi* during the early part of May resulting in a general tendency of both alate and apterous forms to fly and crawl about promiscuously in search of new and less densely populated food plants.

In the majority of fields at Pleasant Valley several species of weeds, including lamb's quarter (*Chenopodium album* L.), clover (*Mellilotus* spp.), purslane (*Portulaca oleracea* L.), and several grasses, along with a number of other plants, succeed in establishing themselves during the early part of the season, especially during periods of heavy rainfall. As our records showed, these plants often became densely populated with various species of aphids. In many cases 50 to 75 *M. pisi* were found on a small clover plant growing in the center of a 20 to 30 acre onion field, and as many as 300 specimens of *A. rumicis* were collected on a small shepherd's purse plant in a similar situation. In most cases, these weeds were cut or pulled by the growers and the plant lice, including nymphs, alate and apterous forms, left in the field with no source of food except the onion plant. In case the weeds were not destroyed early, they soon became overpopulated and served as a center of migration in all directions.

Plant lice apparently have an inherent tendency to make an effort to feed on any plant with which they come in contact and their chief criterion for determining the desirability or undesirability of a plant as a source of food appears to be by sampling it. In other words, the fact that an aphid finds one onion plant an unsatisfactory source of nourishment does

not appear to impress it with the fact that the next onion plant will be of the same nature. As a result, one plant louse in migrating through an onion field may insert its beak and feed to some extent on a number of plants. In one particular instance an alate *M. pisi* was observed to attack 4 onion plants in succession in a period of 30 minutes and at the end of this time was crawling toward another. It was not uncommon to find from a few to 30 or more aphids feeding on a single onion plant near a recently cut weed.

In a field belonging to a grower who had made no effort to control yellow dwarf it was noted that diseased volunteer onions were growing in considerable numbers, as were also a variety of weeds, such as shepherd's purse, (*Capsella bursapastoris* (L.)), seedling box elder plants (*Acer negundo* L.), and others. Many of these were heavily infested with aphids. During the first week of June (1932) the weeds were cut by the grower and an examination of the field a few days later showed that large numbers of aphids were feeding on onions. In order to secure conclusive evidence that aphids become infectious by feeding on diseased plants under field conditions, 115 *A. rumicis*, found on diseased volunteer onions near recently cut shepherd's purse plants, were collected and confined on 6 disease free onions. At the end of two weeks 5 of these had developed typical yellow dwarf symptoms. Following the time at which the weeds were cut frequent field observations showed that many new cases of yellow dwarf had developed with the diseased volunteer onions acting as centers of infection.

Another factor which was observed to be associated with the rate of spread of yellow dwarf was the proximity of the onion field to other vegetation such as alfalfa and certain vegetable crops which, as a rule, supported a heavy aphid population during the spring months. It has been noticed repeatedly that the incidence of yellow dwarf is unquestionably greater in onion fields adjacent to alfalfa or clover than in areas located at some distance from these legumes.

The Pleasant Valley onion growing district is located along the Mississippi River and comprises a narrow valley approximately .75 mile in width and 4 or 5 miles in length. It is bordered on the east by the Mississippi River and on the west by a series of hills or bluffs which, to some extent act as a barrier between the valley and the higher grain growing region to the west.

In the Pleasant Valley district there is, in addition to onions, a considerable acreage of vegetables such as cabbage, melons and other plants which are favorable hosts for aphids. Also, there are rather extensive areas of waste land, partly in the form of ditch banks and low lands near the river, which are unsuitable for cultivation. In such places grow a wide variety of weeds and trees many of which at times become heavily infested with aphids. It is quite evident, therefore, that in this area ecological factors are favorable for the development of heavy aphid populations of various species which, in the course of their wanderings and migrations, may serve as disseminating agents for the yellow dwarf virus.



Approximately 10 miles from Pleasant Valley is a district where from 150 to 200 acres of set onions are produced each year. Although yellow dwarf is known to have been present in this territory since 1928, no special efforts have been made to control the disease and it has not at any time attained such proportions as to cause appreciable commercial losses. Furthermore, during the last few years, there has been a gradual decrease in the percentage of infection and at the present time (1936) only a trace of the disease can be found. Why such a condition should exist in this region is not definitely known but it is apparently related to a scarcity of the insects which disseminate the disease under natural conditions. In contrast to the Pleasant Valley area, this district is located in the grain growing region where practically all of the land is either planted in small grain and corn or used as pasture. Consequently, the plant complex appears to be less conducive to the production of heavy aphid populations of various species during the onion growing season.

At Pleasant Valley, Iowa, commercial onions are grown from both sets and from seed, the latter being originally free of yellow dwarf since there is no transmission through the seed. The more susceptible growing period of seedling onions, however, coincides with the maximum aphid activity resulting in highly favorable conditions for transmission of the virus from set onions, which have carried the disease over from the previous season, to seedling onions grown both for commercial purposes and for sets the coming year. Thus cultural practices in combination with the natural characteristics of plant lice are conducive to a perpetuation of the disease from year to year.

During the 1932 growing season, at Pleasant Valley, there was in general a gradual increase in plant lice until the latter half of June. In August and September, because of parasites and predators along with other factors of the environment, the aphid population was in most cases reduced to inappreciable numbers.

The most extensive migration of any one species was that of *H. atriplicis* which began on June 11 (1932) and covered a period of about 10 days. On June 11 they were present in onion fields, as determined by making counts at various points, at the rate of 10,850 per acre. On June 12 individuals of this species were found to be present in some onion fields at the rate of 52,000 per acre. On June 13 the number of individuals had increased to 87,120 per acre. The constant movement of aphids in an onion field which varies in rapidity among different species undoubtedly brings about a thorough and intricate system of cross inoculation. This condition, combined with the ability of the aphids to obtain the infective principle at one feeding and immediately transfer it to healthy plants, appears to be particularly favorable for a rapid spread of the yellow dwarf virus.

Following the July and August scarcity of plant lice, there was a gradual increase in abundance until freezing temperatures occurred. Coincident with this increase, especially in the latter part of September and early part of October in Iowa, occurs the fall migration of aphids back



to the primary or winter host. The apple grain aphid, *R. prunifoliae*, was especially abundant during the late summer and fall of 1932. On September 19, in onion fields which had been planted in barley after the onion crop was harvested from a dozen to 50 or more individuals of this species could be found on practically every barley plant examined. Many of these were winged forms. At the same time volunteer onions, regardless of whether they were growing nearby or one-half mile or more away from the barley fields, were usually harboring one or more winged forms of this species.

The significance of the heavy late summer and early fall aphid population in connection with yellow dwarf virus dissemination depends entirely upon the importance of volunteer onions in carrying the disease from one season to the next. Evidence that volunteer onion may serve as an over-wintering source for the virus will be presented in a succeeding section of this report.

Further information concerning aphid populations in onion fields was obtained by the use of "screens" consisting of frames, 2 square feet in area, covered over with ordinary 16-mesh screen wire. Over each side of the "screen" was spread a thin layer of "tanglefoot" which served as a trap for any insects, especially smaller ones, which happened to come in contact with it. It was found necessary to replace the "tanglefoot" at about 2-week intervals because of the accumulation of various objects, including wind blown materials such as dust and small particles of trash and miscellaneous species of insects. These "screens" were placed near the center of a large onion field in two different localities designated as locality A and locality B. At locality A the entire "screen" was composed of 4 units (each unit 2'x2' in area), 2 facing north and south, one of which extended from 4 feet above ground surface upward and the other directly above it, and 2 facing west and east with the same arrangement as in the former. At locality B the "screen" was composed of 2 units placed in a manner similar to that in A and extending in height from 6 to 8 feet above the ground level.

Daily examinations of these "screens" were made throughout the growing season of 1932 and records made of the number of aphids trapped and weather conditions, especially the prevailing course of air currents.

Aphids which have been trapped by means of "tanglefoot" are usually in a badly distorted and mutilated condition upon removal from the material, thus making definite identification difficult in many cases. It was comparatively easy, however, to identify the more common and more abundant species. The species most commonly taken were the pea aphid, *M. pisi*, the bean aphid, *A. rumicis*, the melon aphid, *A. gossypii*, and *M. asclepiadis*, a species occurring on milkweed. These species were taken more or less regularly during the spring months. A large number of other species were collected, some of which occasionally were present in considerable abundance. Among these may be mentioned the apple grain aphid, *R. prunifoliae*, the lamb's quarter aphid, *H. atriplicis*, the cabbage aphid, *B. brassicae*, the corn leaf aphid, *A. maidis*.

That the abundance and duration of migration of winged forms is materially influenced by air currents is indicated by the following records made, in connection with the above mentioned "screens", on June 25 and 26. "On June 25 no noticeable wind, 6 aphids collected from screen B, distributed as follows: south 4, north 1, west 1, east 0. June 26 fairly strong breeze from west, 155 aphids collected from screen B, distributed as follows: south 14, north 20, west 121, east 0. These results were duplicated in their essentials throughout the course of the experiment."

On 8 square feet of "screen" located near the center of an onion field comprising approximately 100 acres, broken only by small drainage ditches and field roads, 1697 plant lice, including various species, were collected during the month of June 1932. Based on these figures, which do not take into account the individuals which migrate at heights of less than 4 feet and more than 8 feet, several hundred thousand winged aphids crossed each acre of onion field in the locality under observation during the month of June. It is a fairly well established fact that aphids, whether they be spring, summer, or fall migrants, when influenced by the migratory impulse in combination with the effects of air currents and other environmental factors, exercise little if any selectivity either with respect to direction of movement or duration of flight. Chance therefore seems to be the chief controlling factor operating toward the location of new host plants by the migrating insects.

Four "tanglefoot screens", similar to the ones described above and comprising a total of 10 square feet of surface, were set up in the experimental plots at Ames during April 1933. From these "screens", between April 2 and April 22, inclusive, a total of 2336 aphids of various species were taken. It is of interest to note that there were included among these considerable numbers of individuals of non-migratory species such as *M. alhambri* and *M. asclepiadis*.

These data convincingly indicate that although a species of aphid may be non-migratory, that is, normally confined to one species or group of closely related species of host plants throughout the year for feeding purposes, many winged individuals migrate presumably in search of more favorable food plants of the same species. During their migrations and fortuitous wanderings, these individuals, either by chance or necessity, may rest upon and imbibe the juices of plants which do not serve as the usual host.

#### OVERWINTERING OF YELLOW DWARF VIRUS AND ITS RELATION TO INSECTS

All evidence to date indicates that wild host plants of the onion yellow dwarf virus do not occur in Iowa. Evidence which indicates that it is not a seed borne or soil borne disease also has been presented by Henderson (1935). In view of this situation the "carry over" of the virus from year to year depends entirely on onion bulbs either in storage or as refuse in the field.

## IN STORED BULBS

Each summer during the period of 1928 to 1933 inclusive several hundred bulbs from yellow dwarf infected plants were collected at Pleasant Valley and brought to Ames for use during the following winter and spring as a source of inoculum in insect transmission experiments. From these infected bulbs, which were planted at various times, plants showing unmistakable yellow dwarf symptoms were grown. That onion bulbs, including sets, mother bulbs and cull onions in the field, are an important overwintering source for the yellow dwarf virus has been demonstrated repeatedly.

## IN VOLUNTEER ONIONS

During the fall of 1933, 58 volunteer onion plants in the experimental plots at Ames, were exposed to viruliferous aphids which resulted in 40 successful inoculations, as evidenced by the development of typical disease symptoms. These plants which continued growth as long as weather conditions were favorable remained undisturbed and exposed to normal out-door conditions throughout the winter. The following spring (1934) these plants resumed growth and exhibited typical yellow dwarf symptoms. From aphids confined on these plants 20 healthy plants were inoculated resulting in 13 successful transmissions.

On numerous occasions diseased volunteer onions found growing in the field at Pleasant Valley and at Ames were used as a source of inoculum for infecting healthy plants with successful results. It is, therefore, a well established fact that the virus can overwinter in Iowa in onion bulbs under field conditions.

The "carry over" of the yellow dwarf virus in volunteer onions from year to year is of considerable importance in perpetuating the disease. As a general rule a heavy growth of volunteer onions was produced in the infected area each fall and this coincided to some extent with the fall migration of aphids. During the years 1931-1935 fall volunteer onions were examined and on some occasions from 1 to 15 plant lice were found on practically every plant. These individual aphids, frequently were observed to be feeding on the onion plants. In many cases it was conspicuously noticeable that the percentage of visible yellow dwarf infection in the volunteer onions was higher than in the commercial crop. This increase may have been influenced by the three following factors: (1) masking of symptoms in plants which became infected late in the growing season, and the development of symptoms in these plants during the second growth period, (2) a tendency of yellow dwarf infected bulbs to be less susceptible to a long dormant period, and (3) the heavy population of plant lice which is found in onion fields during the fall migratory period.

## INTER-TRANSMISSIBILITY OF THE YELLOW DWARF VIRUS

A series of experiments were undertaken in an effort to determine whether yellow dwarf could be transmitted to plants other than the



cultivated onion. Colonies of aphids were confined on diseased onion for a period of 24 hours and then transferred to the experimental plants (not onion) for 24 to 48 hours, and in some cases for a considerably longer period. The results of these tests are shown in table 7. It will be noted that of a large number of plants exposed none developed what was considered as yellow dwarf symptoms.

In a number of cases certain of these plants developed a mosaic like appearance suggestive of a virus disease. With all plants listed in table 7 a number of attempts, particularly with those which seemed to exhibit a mosaic appearance, were made to infect healthy onion plants by caging aphids on these plants for a period of time and then transferring them to healthy onions. In no cases, however, was inter-transmissibility demonstrated.

In addition to the plants mentioned in table 7, attempts were made to inoculate a number of other both cultivated and wild plants. Among these were crocus, (*Crocus biflorus*, Mill.), plantain (*Plantago* spp.), corn, (*Zea mays* L.) lamb's quarter (*Chenopodium album* L.), dock, (*Rumex* spp.), alfalfa (*Medicago sativa* L.), milkweed (*Asclepias* spp.),

TABLE 7. Inoculation of plants other than the cultivated onion by means of aphids

| Common name         | Scientific name               | Family         | Number of plants | Number of plants |
|---------------------|-------------------------------|----------------|------------------|------------------|
|                     |                               |                | exposed          | infected         |
| Wild garlic         | <i>Allium canadense</i> L.    | Liliaceae      | 64               | 0                |
| Shallots            | <i>A. " ascalonicum</i> L.    | "              | 37               |                  |
| Leek                | <i>A. " porrum</i> L.         | "              | 16               |                  |
| Garlic              | <i>A. sativum</i> L.          | "              | 10               |                  |
| Chive               | <i>A. schoenoprasum</i> L.    | "              | 24               |                  |
| Field garlic        | <i>A. vineale</i> L.          | "              | 32               |                  |
|                     | <i>Lilium auratum</i> Lindl.  | "              | 6                |                  |
|                     | <i>L. canadense</i> L.        | "              | 2                |                  |
|                     | <i>L. elegans</i> Thunb.      | "              | 2                |                  |
|                     | <i>L. henryi</i> Baker        | "              | 6                |                  |
|                     | <i>L. japonicum</i> Thunb.    | "              | 4                |                  |
| Easter lily         | <i>L. longiflorum</i> Thunb.  | "              | 11               |                  |
|                     | <i>L. regale</i> Wils.        | "              | 4                |                  |
|                     | <i>L. rubrum</i> L.           | "              | 16               |                  |
|                     | <i>L. speciosum</i> L.        | "              | 15               |                  |
|                     | <i>L. superbum</i> L.         | "              | 5                |                  |
|                     | <i>L. tenuifolium</i> Fisch.  | "              | 12               |                  |
| Tiger lily          | <i>L. tigrinum</i> Ker.       | "              | 8                |                  |
|                     | <i>L. umbellatum</i> Pursh.   | "              | 5                |                  |
| Tulip               | <i>Tulipa gesneriana</i> L.   | "              | 8                |                  |
| Jonquil             | <i>Narcissus jonquilla</i> L. | Amaryllidaceae | 26               |                  |
|                     | <i>N. odoratus</i> L.         | "              | 26               |                  |
| Daffodil            | <i>N. pseudo-narcissus</i> L. | "              | 24               |                  |
| Chinese sacred lily | <i>N. tazetta</i> L.          | "              | 17               |                  |
|                     | <i>Iris</i> sp.               | Iridaceae      | 18               |                  |
| Gladiolus           | <i>Gladiolus</i> sp.          | "              | 12               |                  |



and various species of grasses. No evidence was obtained to indicate that any of these plants are susceptible to yellow dwarf.

Healthy onion plants were exposed to the feeding of aphids which had been previously confined on plants (not onion) either known to be infected with a virus disease or exhibiting virus disease like symptoms. Among these were: Easter Lily, (*L. longiflorum*), milkweed, (*Asclepias* sp.), tomato (*Lycopersicum esculentum* Mill.), Tulip (*Tulipa* sp.), cucumber (*Cucumis sativus* L.), alfalfa (*Medicago sativa* L.), aster (*Calistephus chinensis* Nees.), sweet clover (*Mellilotus alba* Desr.), wild lettuce (*Lactuca canadensis* L.), plantain (*Plantago* ssp.), dock (*Rumex* ssp.), purslane (*Portulaca oleracea* L.), corn (*Zea mays*), garden pea (*Pisum sativum* L.), iris (*Iris* sp.), garlic (*Allium sativum* L.), leek (*A. porrum* L.), and a number of others. No definite indications of inter-transmissibility were obtained.

In the Pleasant Valley onion growing district are a number of localities in which wild garlic, commonly referred to as wild onion, (*Allium canadense* L.) grows quite profusely from year to year. It seemed logical to suspect that this closely related species of the cultivated onion might serve as a reservoir of yellow dwarf virus. A large number of experiments were therefore conducted to test this hypothesis through the agency of aphid vectors, but in no case was a relationship established. In some instances, wild garlic plants, which were inoculated by means of aphids that had previously fed on yellow dwarf infected onion plants, developed a mild chlorotic condition somewhat suggestive of a virus disease. Attempts to infect healthy cultivated onion by using such plants as a source of inoculum, however, were unsuccessful. Likewise, 32 plants of field garlic (*Allium vineale* L.) inoculated by means of infective aphids gave negative results.

During the spring of 1932 daily observations were made by the writer in and around onion fields in the infected area for the purpose of locating any wild or cultivated plants which showed mosaic-like symptoms suggestive of yellow dwarf. A number of plants displaying such symptoms were discovered and in practically all instances attempts were made to inoculate healthy onion plants by means of plant lice which had been previously confined on the suspicious plants. No successful transfers were obtained.

In addition to the varieties of onions which are most commonly grown on a commercial scale in the yellow dwarf infected area, consisting mainly of the Red Globe and Yellow Bottleneck strains, yellow dwarf was experimentally transmitted by means of plant lice, in the greenhouse and experimental plots at Ames, to 30 varieties of cultivated onions.

#### DISCUSSION

The distribution of yellow dwarf in Iowa was found to be confined largely to the Pleasant Valley onion growing district, a condition which appeared to be related to cultural practices. In this district a consider-

able proportion of the commercial onion crop was grown from sets, whereas, in other onion growing regions of the state, seed is the principal source of commercial plantings. Evidence to date indicates that the disease is not seed born, thus the principal overwintering source of the virus—namely, bulbs, is eliminated in areas where the commercial crop is produced directly from seed.

Some virus diseases of plants are known to attack only a small number of more or less closely related species while a few have been shown to have a rather wide host range, such as aster yellows and ring spot disease of tobacco. In contrast, information available at the present time indicates that the onion yellow dwarf virus is highly specific and under normal conditions is not known to attack any plant other than the cultivated onion (*Allum cepa* L.). That such a condition should actually exist, however, hardly seems tenable and it is believed that eventually additional host plants will be located either in this country or such other places where the disease may occur.

A correct understanding of the importance of aphids in field dissemination of the onion yellow dwarf virus depends largely upon a knowledge of the life history and habits of aphids in general. The behavior and migration of these insects are governed largely by their host specificities, periodic or seasonal host restrictions, numerical abundance, capacity for reproduction, and availability of food plants. The abundance of aphids varies with weather conditions, with the prevalence of aphidophagous fungi, with the abundance of predacious and parasitic insect enemies, and other factors of the environment. Some species are monophagous, whereas, other forms are polyphagous and feed with varying degrees of success upon many different species of plants.

With favorable food plants, optimum conditions of temperature, humidity, and other biotic conditions during the spring and summer, plant lice multiply rapidly and often thickly populate and overrun their host. This over-crowding, together with the age and varying degrees of succulency of food plants, tends to accelerate migration. In all species, particularly among the winged individuals, there is an inherent urge to wander so that under field conditions migration occurs almost incessantly. Thus, both monophagous and polyphagous species wander about in the spring, summer, and fall in search of new and less densely populated host plants during which they may feed to some extent upon any plant with which they come in contact. The onion seems to serve largely as a place to rest and a temporary source of nourishment during their fortuitous wanderings and migrations. Evidence indicates that such a relationship, however, is sufficient to effect a spread of the virus from diseased to healthy plants.

Plant lice acquire the causal agent during the first feeding upon diseased onion and immediately afterwards are capable of infecting healthy plants, thereby eliminating the hazards that necessarily would be encountered should a prolonged incubation period in the insect be

obligatory. If there is a latent period, it is extremely short and aphids seem to have little, if any, more than an accidental connection with yellow dwarf virus.

The experiments presented in this paper have shown that inoculated aphids retain their infective power for only a relatively short time. Whether this condition is because of an attenuation or destruction of the virus within the insect or owing to a passage through the body and final elimination is not known.

Field observations and experimental data indicate that practically any species of aphid is a potential vector of onion yellow dwarf. In Iowa there are probably more than 300 species of plant lice any one of which may be a contributing factor toward the final results of field dissemination.

It is a well established fact that the virus of yellow dwarf overwinters in diseased sets, mother bulbs, and commercial onions. Such bulbs, together with diseased culls thrown in refuse piles and dump heaps or left in the field, may serve as sources of inoculum for the vectors the following season. Since no host plant other than the cultivated onion has yet been found in Iowa, the diseased onion bulbs appear to be the only reservoir of the overwintering virus in this area.

The control of onion yellow dwarf, in common with other diseases which depend on insects for natural dissemination, is either a matter of breaking the association of the vectors and the disease producing agent or development of resistant or immune varieties. Up to the present time the latter method does not seem to have offered any promise of immediate results. There appears to be little if any prospect of any practical form of general attack upon the aphid vectors being successful in controlling the disease. Thus, the only alternative is to remove or eliminate the source of inoculum which is being accomplished through the use of disease free seed stock grown in non-infected areas in combination with an application of ordinary field sanitation measures.

#### SUMMARY AND CONCLUSIONS

Yellow dwarf is a virus disease of the mosaic type affecting the cultivated onion. In many cases, however, and especially in the advanced stages of the disease, chlorosis is more or less general and suggests the yellows type of disease.

In transmission experiments the virus was transmitted from diseased to healthy plants by 48 species of aphids. All transmission tests with insects, other than aphids, with two doubtful exceptions, gave negative results.

The incubation period of the disease in the plant was found to be relatively short, ranging from 7 to 12 days in most cases with an average of 10.3 days.

It was determined that plant lice acquire the causal agent during the first feeding on diseased onion plants and immediately thereafter are capable of infecting healthy plants.

Experimental evidence convincingly demonstrates that if there is a



period of delayed infectivity in the insect it is extremely short (only a few minutes) and that infective aphids, feeding upon either healthy susceptible hosts, immune hosts, or confined without food become non-viruliferous within the course of a few hours.

Under certain conditions the symptoms of yellow dwarf may be completely masked and not become visibly evident until after the bulbs have undergone a rest period and then regrown. Experiments were conducted in which it was found that such plants, can serve as a source of infection for plant lice, which, when so inoculated, can convey the disease to healthy onions.

Although the onion plant serves as only an incidental or transitory host of aphids in Iowa, field observations made in the infected area show that they are present in sufficient numbers and at the proper time to play an important role in field dissemination of the disease.

Since the only known overwintering source of the virus is in the bulbs, both in storage and in the field, control can be accomplished by destruction of left-over bulbs in the field coupled with planting of disease free sets grown in non-infected areas.

Transmission experiments with a large number of species of plants other than the cultivated onion resulted in no successful transfers. The disease was transmitted to more than 30 varieties of cultivated onions by means of plant lice.

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# STRESSES IN AN ORTHOTROPIC ELASTIC LAYER<sup>1</sup>

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Received January 18, 1940

In an isotropic elastic medium the physical properties of the medium are independent of any selected axes of reference, that is they are the same in all directions. In an anisotropic medium, the elastic moduli are associated with certain types of symmetry and are dependent upon the reference system chosen. In an orthotropic material one may define the elastic moduli to be those associated with three orthogonal planes of symmetry, involving six distinct moduli.

In this paper we are concerned with a problem of plane deformation in which it is assumed that the stressed structure is very long in one direction when compared to its transverse directions. This means that all transverse sections are under the same system of forces and all the deformations lie in this transverse plane. The elastic constants may be reduced to only four independent moduli if we assume that the physical properties of the medium remain the same when the horizontal axes are interchanged.

Consider the orthotropic layer of indefinite extent horizontally and of finite depth  $h$ . The coordinate system is taken with the  $x$  axis in the plane of separation between the elastic layer and the inelastic base. The surface loading,  $q(x)$ , is symmetrical with respect to the  $y$  axis, and is of such a character in the finite portion of the plane that it is capable of being represented by a Fourier integral.

The problem is to determine the stresses and displacements in the elastic layer under surface loads and subject to known boundary conditions at the plane of separation. Two cases will be considered

- (a) The separation plane,  $y = 0$ , is rough,
- (b) The separation plane,  $y = 0$ , is smooth.

From the general results of these cases, one may show that the known results for the corresponding isotropic plane deformation and isotropic plane stress cases may be deduced. The special case of orthotropic plane strain is solved for the semi infinite medium by a limiting process on the results of cases (a) and (b).

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<sup>1</sup>Presented to the American Math. Society, Dec. 2, 1939. This investigation has been carried out under Project No. 14 of the Industrial Science Research Division of Iowa State College by the first of the above authors as Research Fellow in the Department of Mathematics.

## EQUILIBRIUM EQUATION

The linear equalities relating the unit strains to the unit stresses in an orthotropic elastic medium are assumed to be of the form

$$(1) \quad \begin{aligned} \varepsilon_x &= \frac{\sigma_x}{E_x} - \frac{\nu_x \sigma_y}{E_y} - \frac{\nu_x \sigma_z}{E_x}, \\ \varepsilon_y &= \frac{\sigma_y}{E_y} - \frac{\nu_y \sigma_x}{E_x} - \frac{\nu_y \sigma_z}{E_x}, \\ \varepsilon_z &= \frac{\sigma_z}{E_x} - \frac{\nu_x \sigma_x}{E_x} - \frac{\nu_y \sigma_y}{E_y}, \end{aligned}$$

where  $E_x, E_y, \nu_x, \nu_y$  are the compression moduli and contraction ratios for a two dimensional section. In the case of plane deformation, the strain  $\varepsilon_z = 0$  and the first two of Eqs. (1) become

$$(2) \quad \begin{aligned} \varepsilon_x &= \frac{1 - \nu_x^2}{E_x} \sigma_x - \frac{\nu_x(1 + \nu_x)}{E_y} \sigma_y, \\ \varepsilon_y &= \frac{1 - \nu_x \nu_y}{E_y} \sigma_y - \frac{\nu_y(1 + \nu_x)}{E_x} \sigma_x. \end{aligned}$$

From the relation of symmetry with respect to the  $x$  and  $y$  axes the homogeneous form of the strain energy function is

$$(3) \quad 2U = c_{11} \sigma_x^2 + c_{22} \sigma_y^2 + 2c_{12} \sigma_x \sigma_y + c_{44} \tau_{xy}^2.$$

If one compares Eqs. (2) with the strains obtained from Eqs. (3) by the appropriate derivative

$$(4) \quad \varepsilon_x = \frac{\partial U}{\partial \sigma_x}, \quad \varepsilon_y = \frac{\partial U}{\partial \sigma_y}, \quad \gamma_{xy} = \frac{\partial U}{\partial \tau_{xy}},$$

then

$$(5) \quad E_x/E_y = \nu_y/\nu_x = k^2.$$

Designating  $E_x = E$  and  $\nu_x = \nu$ , one finds that Eqs. (2) become

$$(6) \quad \begin{aligned} E \varepsilon_x &= (1 - \nu^2) \sigma_x - k^2 \nu (1 + \nu) \sigma_y, \\ E \varepsilon_y &= k^2 [(1 - k^2 \nu^2) \sigma_y - \nu(1 + \nu) \sigma_x]. \end{aligned}$$

A definition of the shear modulus consistent with known special cases is

$$(7) \quad G = \frac{E}{P + R},$$

where

$$(8) \quad \begin{aligned} P &= k^2 (1 - \nu^2 k^2 + \nu + \nu^2) , \\ R &= 1 - \nu^2 + k^2 \nu + k^2 \nu^2 . \end{aligned}$$

From the usual equilibrium equations a stress function  $\phi$  is defined by

$$(9) \quad \sigma_x = \frac{\partial^2 \phi}{\partial y^2} , \quad \sigma_y = \frac{\partial^2 \phi}{\partial x^2} , \quad \tau_{xy} = - \frac{\partial^2 \phi}{\partial x \partial y} .$$

Inserting the strain components when expressed in terms of  $\phi$  into the compatibility equation, one obtains the differential equation of equilibrium,

$$(10) \quad K^2 \frac{\partial^4 \phi}{\partial x^4} + (1 + K^2) \frac{\partial^4 \phi}{\partial x^2 \partial y^2} + \frac{\partial^4 \phi}{\partial y^4} = 0$$

with

$$(11) \quad K^2 (1 - \nu^2) = k^2 (1 - \nu^2 k^2) .$$

One seeks a solution  $\phi$  of Eq. (10) which can be adjusted to meet the boundary conditions. Such a solution is

$$(12) \quad \phi = \int_0^\infty \{A \cosh \alpha y + B \sinh \alpha y + C \cosh K \alpha y + D \sinh K \alpha y\} \cos \alpha x \, d\alpha$$

in which A, B, C, and D are constants. Before these constants can be determined so that the boundary conditions are satisfied, the following relations are required. From Eq. (9),

$$(13) \quad \begin{aligned} \sigma_x &= \int_0^\infty \{A \cosh \alpha y + B \sinh \alpha y + CK^2 \cosh K \alpha y + \\ &\quad DK^2 \sinh K \alpha y\} \alpha^2 \cos \alpha x \, d\alpha , \\ \sigma_y &= - \int_0^\infty \{A \cosh \alpha y + B \sinh \alpha y + C \cosh K \alpha y + \\ &\quad D \sinh K \alpha y\} \alpha^2 \cos \alpha x \, d\alpha , \\ \tau_{xy} &= \int_0^\infty \{A \sinh \alpha y + B \cosh \alpha y + CK \sinh K \alpha y + \\ &\quad DK \cosh K \alpha y\} \alpha^2 \sin \alpha x \, d\alpha . \end{aligned}$$

The displacements,  $u$  and  $v$ , are found from Eqs. (6)

$$(14) \quad \begin{aligned} E \frac{\partial u}{\partial x} &= E \varepsilon_x = (1 - \nu^2) \phi_{yy} - k^2 \nu^2 (1 + \nu) \phi_{xx} , \\ E \frac{\partial v}{\partial y} &= E \varepsilon_y = k^2 (1 - \nu^2 k^2) \phi_{xx} - k^2 \nu (1 + \nu) \phi_{yy} . \end{aligned}$$



Since the function  $\phi$  is valid in the region  $0 \leq y \leq h$ , with  $u$  and  $v$  vanishing at infinite values of  $x$ , one may show that these displacements are given by

$$\begin{aligned} Eu &= \int_0^\infty \{R(A \cosh \alpha y + B \sinh \alpha y) + P(C \cosh K\alpha y + \\ &\quad D \sinh K\alpha y)\} \alpha \sin \alpha x \, d\alpha, \\ (15) \quad Ev &= - \int_0^\infty \{P(A \sinh \alpha y + B \cosh \alpha y) + KR(C \sinh K\alpha y + \\ &\quad D \cosh K\alpha y)\} \alpha \cos \alpha x \, d\alpha. \end{aligned}$$

#### CASE (a), ROUGH RIGID BASE

The plane of separation at the base is considered to be such that the tangential shearing forces prevent any lateral movement at the rigid base. The normal loading at the top surface has no tangential shearing forces. These conditions are

$$(16) \quad v \Big|_{y=0} = 0, u \Big|_{y=0} = 0, \tau_{yx} \Big|_{y=h} = 0, -\sigma_y \Big|_{y=h} = \text{normal load.}$$

Since the first three boundary conditions of Eqs. (16) vanish identically in  $x$ , one finds three relations among the constants

$$(17) \quad A = \frac{-P}{R} C = \frac{-PN}{RM} B = \frac{KN}{M} D,$$

with

$$M = P \sinh \alpha h - KR \sinh K\alpha h,$$

$$(18) \quad N = R \cosh \alpha h - P \cosh K\alpha h.$$

From the form of the second of Eqs. (13) the fourth boundary condition in Eq. (16) requires that the normal surface loading,  $q(x)$ , shall be represented in Fourier integral form,

$$q(x) = \frac{2}{\pi} \int_0^\infty \cos \alpha x \, d\alpha \int_0^\infty q(\lambda) \cos \alpha \lambda \, d\lambda = \int_0^\infty Q(\alpha) \alpha^2 \cos \alpha x \, d\alpha$$

where

$$(19) \quad Q(\alpha) = \frac{2}{\pi \alpha^2} \int_0^\infty q(\lambda) \cos \alpha \lambda \, d\lambda.$$

This last boundary condition leads to

$$Q(\alpha) = A \cosh \alpha h + B \sinh \alpha h + C \cosh K\alpha h + D \sinh K\alpha h,$$

which, by Eqs. (17) yields

$$A = \frac{PKNQ(\alpha)}{\Delta},$$

with

$$(20) \quad \Delta = KN(P \cosh ah - R \cosh Kah) - M(KR \sinh ah - P \sinh Kah).$$

The final forms of the results giving the stresses and deflections in the elastic layer for case (a) are the following:

$$\begin{aligned} \sigma_x &= \int_0^\infty \frac{Q(\alpha)}{\Delta} \{NK(P \cosh \alpha y - RK^2 \cosh Kay) - MK(R \sinh \alpha y - \\ &\quad - PK \sinh Kay)\} \alpha^2 \cos \alpha x \, d\alpha, \\ \sigma_y &= - \int_0^\infty \frac{Q(\alpha)}{\Delta} \{NK(P \cosh \alpha y - R \cosh Kay) - M(KR \sinh \alpha y - \\ &\quad - P \sinh Kay)\} \alpha^2 \cos \alpha x \, d\alpha, \\ (21) \quad \tau_{xy} &= \int_0^\infty \frac{Q(\alpha)}{\Delta} \{NK(P \sinh \alpha y - RK \sinh Kay) - MK(R \cosh \alpha y - \\ &\quad - P \cosh Kay)\} \alpha^2 \sin \alpha x \, d\alpha, \\ v &= - \frac{1}{E} \int_0^\infty \frac{Q(\alpha)}{\Delta} \{NK(P^2 \sinh \alpha y - KR^2 \sinh Kay) - MKRP(\cosh \alpha y - \\ &\quad - \cosh Kay)\} \alpha \cos \alpha x \, d\alpha, \\ u &= \frac{1}{E} \int_0^\infty \frac{Q(\alpha)}{\Delta} \{NKPR(\cosh \alpha y - \cosh Kay) - M(R^2K \sinh \alpha y - \\ &\quad - P^2 \sinh Kay)\} \alpha \sin \alpha x \, d\alpha. \end{aligned}$$

#### CASE (b), SMOOTH RIGID BASE

When the inelastic base is frictionless, the tangential stress  $\tau_{xy}$  vanishes at  $y = 0$ . This boundary condition takes the place of the first condition of Eq. (16) in the problem of the rough base. With this and the remaining conditions of Eq. (16), the values of the constants for the stress function  $\phi$  of Eq. (12) are

$$\begin{aligned} B &= D = 0, \\ A\Box &= Q(\alpha)K \sinh Kah, \\ C\Box &= -Q(\alpha) \sinh ah, \end{aligned}$$

with

$$\Box = K \cosh ah \sinh Kah - \sinh ah \cosh Kah.$$

The stresses and displacements for case (b) are:

$$\sigma_x = \int_0^\infty \frac{Q(\alpha)}{\Box} \{K \sinh Kah \cosh \alpha y - K^2 \sinh ah \cosh Kay\} \alpha^2 \cos \alpha x \, d\alpha,$$

$$\sigma_y = - \int_0^{\infty} \frac{Q(\alpha)}{\square} \{K \sinh K a h \cosh \alpha y - \sinh a h \cosh K \alpha y\} \alpha^2 \cos \alpha x \, d\alpha ,$$

(22)

$$\tau_{xy} = \int_0^{\infty} \frac{Q(\alpha)}{\square} \{K \sinh K a h \sinh \alpha y - K \sinh a h \sinh K \alpha y\} \alpha^2 \sin \alpha x \, d\alpha ,$$

$$v = - \frac{1}{E} \int_0^{\infty} \frac{Q(\alpha)}{\square} \{PK \sinh K a h \sinh \alpha y - KR \sinh a h \sinh K \alpha y\} \alpha \cos \alpha x \, d\alpha ,$$

$$u = \frac{1}{E} \int_0^{\infty} \frac{Q(\alpha)}{\square} \{RK \sinh K a h \cosh \alpha y - P \sinh a h \cosh K \alpha y\} \alpha \sin \alpha x \, d\alpha .$$

## SPECIAL CASES

*Types of surface loads.*

In order to evaluate any of the values in Eqs. (21) and (22) the transform  $Q(\alpha)$ , given by Eq. (19), must be obtained for the given symmetrical loading,  $q(x)$ . The following types are given.

(a)  $Q(\alpha) = \frac{-2q_0}{\pi a \alpha^4} \{\cos \alpha(a+b) - \cos \alpha b\}$ , ——— isosceles trapezoid,

(b)  $Q(\alpha) = 2q_0/\pi a \alpha^4 \{1 - \cos \alpha a\}$ , ————— isosceles triangle,

(23)

(c)  $Q(\alpha) = 2q_0/\pi a^3 \{\sin \alpha b\}$ , ————— rectangle,

(d)  $Q(\alpha) = W/\pi \alpha^2$ , ————— concentrated load  $W$ ,

(e)  $Q(\alpha) = q_0/2\beta \{\sqrt{\pi} e^{-\alpha^2/4\beta^2}\}$ , ————— normal distribution loading.

In each of the preceding cases,  $q_0$  is the maximum ordinate of the distributed loading. In (a) the isosceles trapezoid has its bases  $2(a+b)$  and  $2b$ ; the isosceles triangle of (b) is the special case of (a) when  $b$  is zero, and the rectangle of (c) is the special case of (a) when  $a$  is zero. The concentrated load of (d) is a limiting case of (c). The normal distribution loading is represented in type (e).

*Semi infinite layer.*

The results for a semi infinite orthotropic layer in plane strain may be derived from either of the preceding cases by translating the origin to the top surface, allowing  $h$  to increase indefinitely and reversing the

direction of the  $y$  axis. The limiting value of the stress  $\sigma_x$  in the first of Eqs. (22) becomes

$$\begin{aligned}\sigma_x &= \lim_{h \rightarrow \infty} \int_0^{\infty} Q(\alpha) \left\{ \frac{K e^{-K\alpha h} - \alpha h - \alpha y - K^2 e^{-K\alpha h} - \alpha h - K\alpha y}}{K e^{-\alpha h} - K\alpha h - e^{-\alpha h} - K\alpha h} \right\} \alpha^2 \cos \alpha x \, d\alpha \\ &= \frac{K}{K-1} \int_0^{\infty} Q(\alpha) \{ e^{-\alpha y} - K e^{-K\alpha y} \} \alpha^2 \cos \alpha x \, d\alpha.\end{aligned}$$

Similarly

$$\sigma_y = \frac{1}{K-1} \int_0^{\infty} Q(\alpha) (K e^{-\alpha y} - e^{-K\alpha y}) \alpha^2 \cos \alpha x \, d\alpha,$$

(24)

$$\begin{aligned}\tau_{xy} &= \frac{K}{K-1} \int_0^{\infty} Q(\alpha) (e^{-\alpha y} - e^{-K\alpha y}) \alpha^2 \sin \alpha x \, d\alpha, \\ Eu &= \frac{1}{K-1} \int_0^{\infty} Q(\alpha) (R K e^{-\alpha y} - P e^{-K\alpha y}) \alpha \sin \alpha x \, d\alpha, \\ Ev &= \frac{-K}{K-1} \int_0^{\infty} Q(\alpha) (P e^{-\alpha y} - R e^{-K\alpha y}) \alpha \cos \alpha x \, d\alpha.\end{aligned}$$

*Isotropic plane deformation.*

When  $k^2 = 1$  all the preceeding results reduce to those for plane strain or plane deformation in an isotropic medium. Eqs. (21) then become those obtained by several writers<sup>1</sup>. The isotropic case for a smooth base is obtained from Eqs. (22) and confirm the results found by Melan<sup>2</sup>. The isotropic semi infinite case is deduced from Eqs. (24) by the limiting values when  $K \rightarrow 1$  and yields the results of Boussinesq. For example

$$\sigma_x = \int_0^{\infty} Q(\alpha) e^{-\alpha y} (1 + \alpha y) \alpha^2 \cos \alpha x \, d\alpha.$$

(25)

$$\sigma_x = \frac{-P x^2 y}{\pi (x^2 + y^2)^2}$$

for the particular case of a concentrated load  $P$  at the origin.



*Relation with plane stress.*

In plane stress or generalized plane stress  $\sigma_z = 0$ . By comparing Eqs. (1) with  $\sigma_z = 0$  with the corresponding Eqs. (2), one may make the transition from plane stress to plane displacement by replacing

$$E_x \sim E_x / (1 - \nu_x^2) \quad , \quad \nu_x \sim \nu_x (1 + \nu_x) / (1 - \nu_x \nu_y), \quad (26)$$

$$E_y \sim E_y / (1 - \nu_x \nu_y) \quad , \quad \nu_y \sim \nu_y / (1 - \nu_x).$$

H. Okubo<sup>3</sup> has solved the generalized plane stress problem for case (b) employing the shear modulus

(27)

$$G = \frac{E_x E_y}{E_x + E_y (1 + 2\nu_y)}.$$

By the substitutions of Eqs. (26) one may readily show that  $G$  of Eq. (27) becomes  $G$  of Eq. (7). The fact that the stresses in Eqs. (22) may be derived from the corresponding results of Okubo by the substitutions of Eqs. (26) has been confirmed. The corresponding results for the *Orthotropic Plane Stress Problem of Case (a)* are the following:

$$\sigma_x = k \int_0^\infty \frac{Q(\alpha)}{\Delta_1} \{ N_1 (P_1 \cosh \alpha y - R_1 k^2 \cosh k \alpha y) - M_1 (R_1 \sinh \alpha y - P_1 k \sinh k \alpha y) \} \alpha^2 \cos \alpha x \, d\alpha,$$

$$\sigma_y = - \int_0^\infty \frac{Q(\alpha)}{\Delta_1} \{ N_1 k (P_1 \cosh \alpha y - R_1 \cosh k \alpha y) - M_1 (R_1 k \sinh \alpha y - P_1 \sinh k \alpha y) \} \alpha^2 \cos \alpha x \, d\alpha,$$

(28)

$$\tau_{xy} = k \int_0^\infty \frac{Q(\alpha)}{\Delta_1} \{ N_1 (P_1 \sinh \alpha y - R_1 k \sinh k \alpha y) - M_1 (R_1 \cosh \alpha y - P_1 \cosh k \alpha y) \} \alpha^2 \sin \alpha x \, d\alpha,$$

$$E\nu = -k \int_0^\infty \frac{Q(\alpha)}{\Delta_1} \{ N_1 (P_1^2 \sinh \alpha y - k R_1^2 \sinh k \alpha y) - M_1 R_1 P_1 (\cosh \alpha y - \cosh k \alpha y) \} \alpha \cos \alpha x \, d\alpha,$$

$$Eu = \int_0^\infty \frac{Q(\alpha)}{\Delta_1} \{ N_1 P_1 R_1 k (\cosh \alpha y - \cosh k \alpha y) - M_1 (R_1^2 k \sinh \alpha y - P_1^2 \sinh k \alpha y) \} \alpha \sin \alpha x \, d\alpha,$$

where

$$P_1 = k^2(1 + \nu),$$

$$R_1 = 1 + \nu k^2,$$

$$M_1 = P_1 \sinh ah - R_1 k \sinh kah,$$

$$N_1 = R_1 \cosh ah - P_1 \cosh kah,$$

$$\Delta_1 = kN_1(P_1 \cosh ah - R_1 \cosh kah) - M_1(kR_1 \sinh ah - P_1 \sinh kah).$$

The case of plane stress, Eqs. (28), reduces to the case of plane strain, Eqs. (21), by the use of Eqs. (26). When  $k \rightarrow 1$  it can be shown that Eqs. (28) reduce to the results obtained by Marguerre.<sup>4</sup>

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# EFFECT OF pH ON THE TOXICITY OF NICOTINE INJECTED INTO THE COCKROACH PERIPLANETA AMERICANA (L)<sup>1</sup>

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Received February 7, 1940

When organisms, tissues or cells are separated by intact surface membranes from an outside solution containing alkaloid molecules and ions, the molecules generally enter the protoplasm more rapidly than the ions. If, however, the solutions are introduced by injection or other means, directly beneath the body surfaces of organisms, or beneath tissue or cell membranes, physiological action is usually more rapid, and differences between the response to molecules and ions are often less evident. The results obtained by investigators of this subject, however, are not in agreement; some contend that there is no difference between the effects of injected alkaloid molecules and ions, others that a pronounced difference is manifest.

The investigation reported here is an attempt to show whether there is a difference in the reactions of cockroaches to molecular and ionic nicotine when solutions of various pH values are injected into the body cavities of these insects<sup>3</sup>.

## PLAN OF EXPERIMENTS

Two types of experiment were employed in this investigation. The first was designed to test the mortality resulting from injection of a series of relatively large doses of nicotine at three hydrogen ion concentrations. The criterion of death was the inability of the injected insects to crawl 24 hours after treatment. This criterion proved to be a reliable estimate of death or recovery, for the roaches that did not recover completely in 24 hours invariably failed to recover later.

Since speed of toxic action could not be evaluated from the above experiments, a second type of experiment was planned for this purpose. Sublethal doses of nicotine at three hydrogen ion concentrations were injected into roaches, and the times elapsing between treatment and complete paralysis of the legs, and between treatment and recovery of the legs were observed.

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<sup>1</sup> Journal Paper No. J-763 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 137.

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<sup>3</sup> A preliminary account of this investigation has already appeared (7). The present article gives the experimental basis for the conclusions reached, and includes a more critical analysis of the data.



## MATERIALS AND METHODS

The nicotine used in these experiments was a redistilled product which contained 99 per cent of the alkaloid. The pH of the nicotine solutions was determined electrometrically by means of a glass electrode apparatus. Solutions with pH values lower than that of the base were obtained by adding to the latter the necessary quantity of 0.01 M sulphuric acid solution.

Only active adult cockroaches, *Periplaneta americana* (L.) were employed. The 233 males weighed from 0.55 gram to 1.47 grams, the mean weight being  $0.89 \pm 0.01^4$  gram; the 490 females weighed from 0.65 gram to 1.72 grams with a mean weight of  $1.05 \pm 0.01$  grams.

The injections were made with a 0.1 cc. micropipette calibrated in 0.01 cc., the delivery end of which was drawn out to a needle-like point. To the opposite end of the pipette was attached a short length of rubber tubing which served as a mouthpiece. This type of pipette eliminates unnecessary connections in which gas bubbles are likely to form and interfere with quantitative deliveries.

Injection was accomplished by holding the roach loosely between the thumb and first two digits, ventral side up, with the anterior end of the insect directed toward the worker. One of the posterior legs was held in a bent position between the side of the thumb and the index finger, thus exposing the conjunctiva at the distal end of the coxa, into which the injection was made. When the injection was correctly performed, no liquid escaped from the wound; if, however, liquid did escape, the roach was discarded.

Each roach was injected with 0.05 cc. of nicotine solution, the temperature of which was  $23^\circ \pm 1^\circ$  C. After injection, the insect was weighed and placed in a small glass cage for observation.

## RESULTS OF MORTALITY EXPERIMENTS

Solutions of 5 nicotine concentrations were injected into roaches in the manner described. The pH values for the solutions were: 0.05 M pH 9.3 (base), 7.4 and 2.8; 0.02 M pH 9.2 (base), 7.2 and 2.6; 0.013 M pH 9.1 (base), 5.0 and 3.0; 0.01 M pH 9.0 (base), 7.5 and 3.0; 0.0075 M pH 8.8 (base only). Such concentrations of nicotine produce paralysis of the legs almost immediately after injection.

Control roaches injected with 0.05 cc. of distilled water showed no harmful effects, except, perhaps, a slight sluggishness which passed off in a short time. Furthermore, roaches injected with 0.05 cc. of a sulphuric acid solution, equal in acid concentration to that of the most acid nicotine sulphate solution employed, exhibited a noticeable irritation of the injected leg, but suffered no prolonged harmful effect.

Each injection increased considerably the liquid volume of the insect's body. The blood volume of *P. americana* is not known, but that of a smaller related species, *P. fuliginosa* (Serville), is about 0.069 gram

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<sup>4</sup>Standard error of the mean.

per gram of body weight (24). If, as appears reasonable, *P. americana* possesses a similar relative blood volume each injection added about 75 per cent to the normal liquid content of the insect's body, and assured rapid diffusion of the injected nicotine.

The results from the male and female roaches are given in figures 1 and 2 respectively. The mean percentage of mortality for each sex was

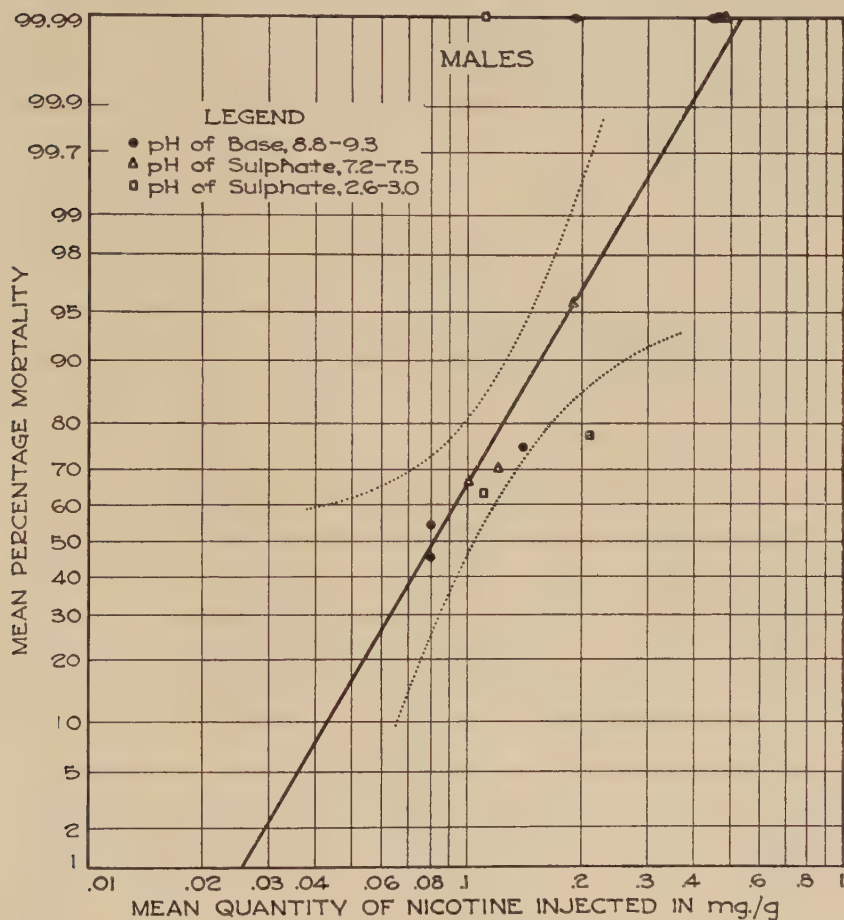


FIG. 1. Mortality curve for males. Each point on the curve represents the mean percentage mortality of a group of 5 to 23 insects. Each insect was injected with 0.05 cc. of nicotine solution.

first determined for each nicotine concentration injected without regard to the pH value; the doses of nicotine were calculated as the mean quantity injected in milligrams per gram of body weight, again disregarding the pH of the solutions. These values were then used to calculate a regression line by the method of Bliss (2). The chi-square test showed that

the fit of the observed to the calculated values was adequate. In figures 1 and 2, the regression lines, which constitute a least mean square fit to the observed mean values, are drawn on logarithmic-probability paper, the plotted points being the observed mean percentages of mortality corresponding with the mean doses of injected nicotine for each pH value. Since the weights of the roaches varied among the samples, the observed doses of injected nicotine for a given pH level are not quite equal.

The toxicity data for the males are not arranged in a definite manner corresponding with the pH values of the solutions, but are quite random. Furthermore, most of the points in the central portion of the graph (fig. 1) lie within a zone, described by dotted lines on either side of the regression line, in which the chances of random occurrence are 95 in 100. The varia-

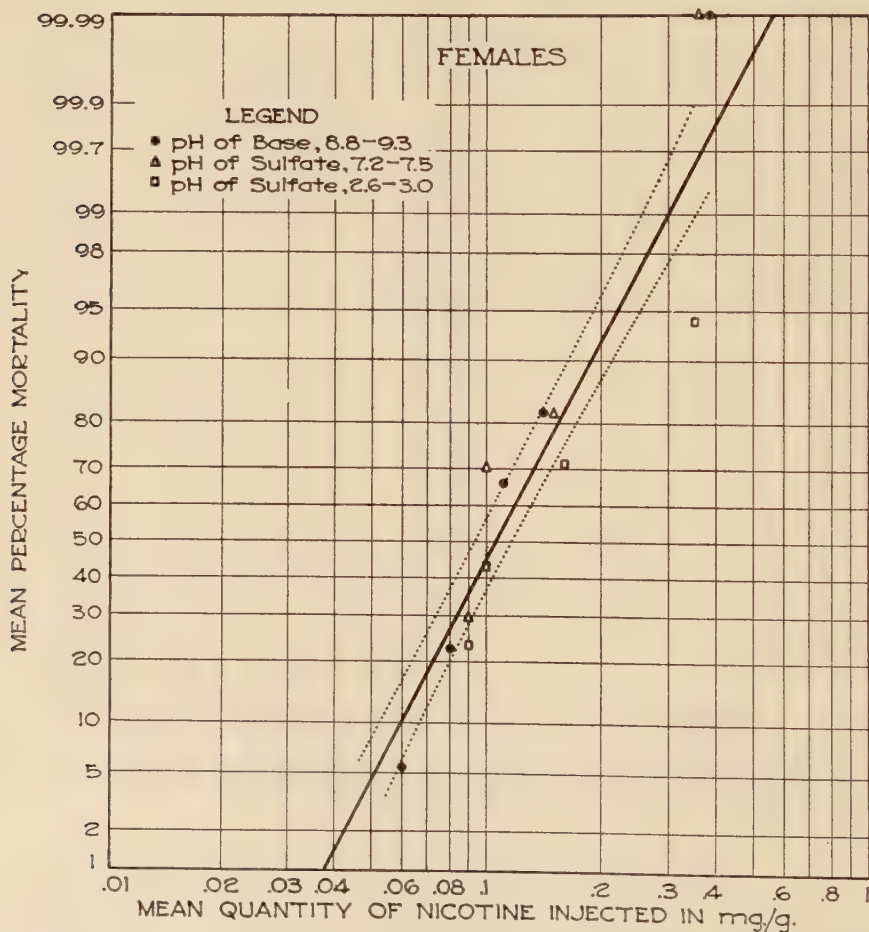


FIG. 2. Mortality curve for females. Each point on the curve represents the mean percentage mortality of a group of 15 to 48 insects. Each insect was injected with 0.05 cc. of nicotine solution.

tion in the percentages of mortality in this population appears therefore to be due to chance rather than to differences in the hydrogen ion concentration of the injected solutions.

The mortality data for the female roaches (fig. 2) were treated in the same manner. The experimentally determined points do not lie so closely to the regression line as those in figure 1, since in the region of 1 to 95 per cent mortality 4 of the points lie well outside the zone in which the chances of occurrence on the basis of random sampling are 95 in 100. Furthermore, 3 of these points represent the lowest mortality and lowest pH value for their respective levels of nicotine dosage. If this indicated an actual association of low toxicity with low pH, one would expect the percentages of mortality for the intermediate pH values (pH 7.2-7.5) to occupy an intermediate position. This is not the case, however; the mortality points for the intermediate pH values are quite random with respect to position between the high and low pH values at any level of nicotine dosage.

A comparison of figures 1 and 2 reveals a sex difference in mortality. The line for the females is displaced somewhat to the right of the line for the males, indicating that the female roaches were more resistant than the males to the injected nicotine. The median dosage in milligrams per gram of body weight for 50 per cent mortality is 0.107 for the females and 0.084 for the males.

#### RESULTS OF PARALYSIS EXPERIMENTS

Cockroaches were injected with solutions of 0.001 M nicotine base, pH 8.3, and with nicotine sulphate solutions of the same molar alkaloid concentration, but with pH values of 6.7 and 2.8. Forty roaches were used in testing each solution, each insect receiving 0.05 cc. of a solution as in the previous experiments. This concentration was not sufficient to cause death, yet it usually produced complete paralysis of the legs which passed off after a time. In some cases, however, the first pair of legs did not become paralyzed.

Since injection was made into the base of one member of the third (posterior) pair of legs, paralysis of this pair occurred first. Then the second and first pairs of legs became paralyzed in the order given. The results of these tests for the males and females are shown in figures 3 and 4 respectively.

No constant differences were found between the pairs of legs, either in paralytic or recovery time, which could be related to the pH of the solutions. Since a sex difference was observed in the mortality experiments, and furthermore, since the mean values plotted in figures 3 and 4 might obscure important differences, a more sensitive analysis of the data was undertaken. For this purpose, the data for the second pair of legs were chosen because the action of nicotine on these appendages was most uniform and complete (table 1). The data for the first pair of legs were ruled out owing to occasional failure of paralysis; that of the third pair because paralysis of the injected member was sometimes prolonged



TABLE 1. *Effect of pH on paralytic and recovery times of cockroaches injected with nicotine*

| pH of* solutions | No. males injected | No. females injected | Mean time to paralysis Secs. |         | Mean recovery time Mins. |         |
|------------------|--------------------|----------------------|------------------------------|---------|--------------------------|---------|
|                  |                    |                      | Males                        | Females | Males                    | Females |
| 8.3**            | 13                 | 27                   | 25.7                         | 23.7    | 12.5                     | 13.0    |
| 6.7              | 10                 | 30                   | 23.8                         | 21.6    | 13.8                     | 13.0    |
| 2.8              | 17                 | 23                   | 19.9                         | 21.5    | 14.7                     | 13.1    |

\* Each insect was injected with 0.05 cc. of a 0.001 M nicotine solution into the base of a member of the posterior pair of legs.

\*\* Free base.

and readings were recorded only from the uninjected member. The problem was one of analyzing the variance in a table of multiple classification. The mathematical requirements of such a problem demand that the frequencies of the sexes be equal or at least proportional. The experiments were well under way, however, before the possibility of a sex difference was realized, and the numbers of male and female insects finally used were neither equal nor proportional. A method for the analysis of

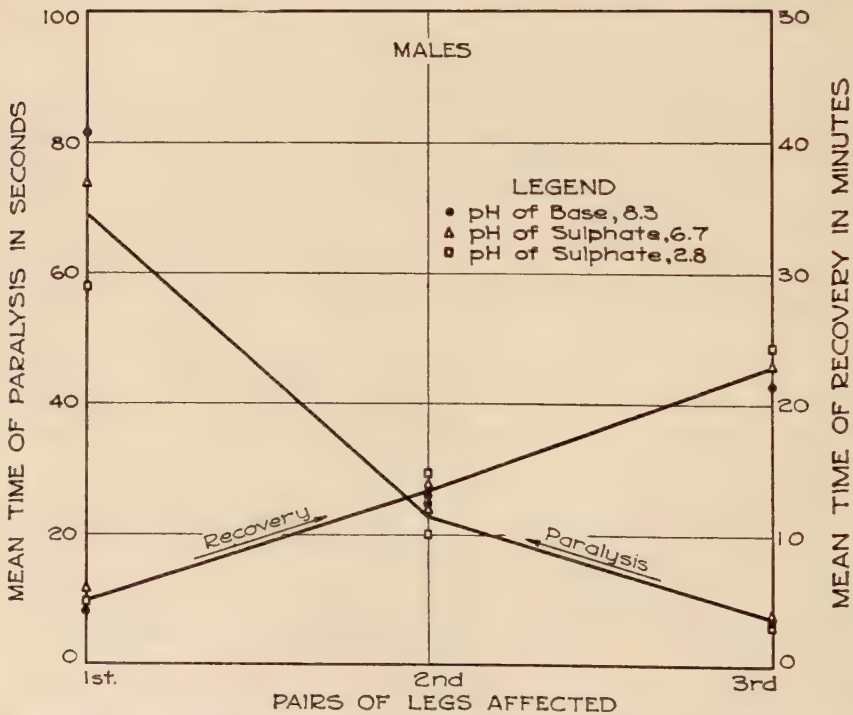


Fig. 3. Speed of paralysis and recovery of the legs of male cockroaches after each insect was injected with 0.05 cc. of a 0.001 M nicotine solution.

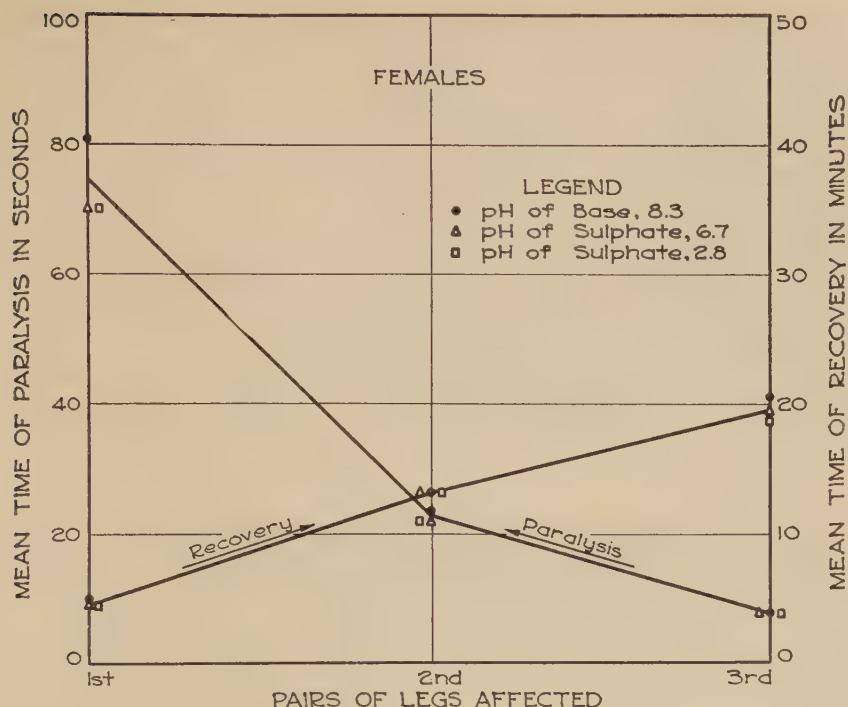


Fig. 4. Speed of paralysis and recovery of the legs of female cockroaches after each insect was injected with a 0.05 cc. of a 0.001 M nicotine solution.

disproportionate subclass numbers in a table of multiple classification (Snedecor and Cox, 22) was therefore chosen. Calculation of  $\chi^2$ , to test the agreement of the actual with the expected numbers, gave a value which corresponded with a probability of 0.20 indicating a reasonable approach of the actual to the expected proportional numbers.

TABLE 2. Analysis of variance of paralytic and recovery times of cockroaches injected with nicotine

| Source of variation               | Degrees of freedom | Mean square                 |                         |
|-----------------------------------|--------------------|-----------------------------|-------------------------|
|                                   |                    | For time to paralysis Secs. | For recovery time Mins. |
| Between means of subclasses ..... | 5                  | 65.91                       | 8.47                    |
| Between means of sexes .....      | 1                  | 20.88                       | 10.48                   |
| Between means of pH values .....  | 2                  | 115.18**                    | 6.57                    |
| Interaction .....                 | 2                  | 39.15                       | 9.36                    |
| Within subclasses .....           | 114                | 23.96                       | 8.97                    |

\*\* Highly significant difference.

The analysis of the mean times for paralysis of the second pair of legs is summarized in table 2. The mean square of the variation between pH of the solutions is significantly larger than the mean square of the variation within the subclasses. Table 1 shows that a decrease in mean paralytic time for both males and females accompanies the decrease in pH of the solutions. The greatest differences are found in the column for the males; but the trend of the values for the females is in the same direction. The results are definitely against the hypothesis that injected alkaloid molecules are more active in the cockroach than injected ions. It is believed that these results represent the slightly accelerating effect on paralysis of the sulfuric acid in the solutions. A difference in paralytic time, associated with sex, is not indicated by this analysis.

Table 2 also presents the analysis of the mean times for recovery of the second pair of legs. No significant differences attributable either to pH of solutions or to sex are indicated by this analysis.

#### DISCUSSION

The solutions injected varied considerably with respect to the proportion of molecules and ions. Percentage dissociation was calculated from the equation,  $\log \alpha/1-\alpha = (pK_w - pK_b) - \text{pH}$ , in which  $pK_w$  for 23°C. is 13.96 (Clark, 4),  $pK_b$  6.046 (Craig and Hixon, 5) the values of  $\alpha$  equivalent to  $\alpha/1-\alpha$  being obtained from Clark's table (4, p. 677). For solutions of pH 9.3 to 8.8, dissociation ranges from about 4 to 12 per cent, for those of pH 7.5 to 7.2 about 72 to 81 per cent, for those of pH 6.7 to 5.0 about 92 to 99.9 per cent; while in solutions of less than pH 5.0, the nicotine is almost completely ionized. Yet notwithstanding the diversity in molecular and ionic content, the toxic and paralytic effects produced by solutions of equal nicotine concentration were the same or very similar.

The effects of nicotine by injection have been studied previously on several species of insects. Michalsky (17) injected solutions of various concentration into cockroaches, observing a progressive paralysis of the legs closely resembling that described in this paper. Becker (1) described the effects produced by injecting nicotine base into the pericardial cavities, head ampullae and intestines of grasshoppers. Campbell (3) determined the median lethal dose of nicotine base by injection for the fifth instar silkworm (*Bombyx mori* L.) as about 0.0015 milligram per gram of body weight. Levine and Richardson (13) observed the synergistic effect of some inorganic salts on the paralytic action of nicotine base when injected into the posterior leg of *Periplaneta americana*. None of these workers concerned themselves with the pH of the injected solutions.

Two papers, which relate to the effect of the pH of injected nicotine on insects, have been found in the literature. Hockenyos and Lilly (12) injected nicotine sulphate solutions of various pH values into larvae of the white-lined sphinx, *Celerio lineata* Fab. The observed differences in effect were slight. McIndoo (15) administered nicotine and nicotine sulphate solutions to adults of the black blowfly, *Phormia regina* Meig., each

fly receiving 1.25 cu. mm. of a solution which contained 0.25 per cent nicotine. The pH of the solutions was not recorded. The criteria of effect were "first reaction time," the period after injection before appearance of the first signs of life, and "revival time," or the period after injection before the insect was able to get upon its feet. With these criteria as a basis, nicotine proved significantly more toxic to the females than nicotine sulphate. The data for the males were less extensive and the differences less striking, yet they pointed in the same direction. *Phormia* males were always more susceptible to injected nicotine than females of the same age.

Investigators are not agreed with respect to the relative effect of the free bases and salts of alkaloids when they are injected into vertebrate animals. Moore and Row (18) compared the toxic action of free nicotine and nicotine hydrochloride on the frog after subcutaneous injection, muscular paralysis and blood pressure furnishing the indexes of toxicity. The free base was noticeably more toxic than the salt; and the authors concluded that the former is alone responsible for the observed effects, the salt becoming active only as the base is liberated from it by reaction with the blood and lymph. The results of Macht and Craig (14) from the intraperitoneal injection of the green frog with free nicotine and nicotine hydrochloride confirm those of Moore and Row. Paralysis and characteristic posture were employed as criteria of effect. In addition, Macht and Craig found nicotine base more toxic than the hydrochloride when injected intramuscularly and intraperitoneally into rats and mice, and intravenously into cats, survival time being the criterion of effect. With reference to these results, the authors comment as follows: "The marked difference between the alkaloid nicotine and its salts is especially interesting when compared with the relative effects of certain other alkaloids and their salts, which were used as controls. We have made comparative pharmacological studies with solutions of atropine and atropine sulphate, cocaine and cocaine hydrochloride and strychnine (alcoholic solutions) and strychnine sulphate and found that those alkaloids did not differ substantially in toxicity from their salts."

Some other investigators have failed to show a significant difference in the action of alkaloid bases and salts after injection into vertebrate animals. Sollmann (23) observed that the anesthetic properties of cocaine hydrochloride and novocaine<sup>5</sup> were not increased by the addition of sodium bicarbonate when solutions of these alkaloids were injected subcutaneously in man. Meeker (16) obtained similar results from endermic injection of novocaine into man. Dierks (6) states that alkaloid salts, by subcutaneous injection or by administration *per os*, maintain the same activity either with or without the addition of sodium bicarbonate. Gwinn and Ferber (10) found that alkaline and acid solutions of novocaine had exactly the same effect on the cat when administered by rapid intravenous injection. However, in mice by subcutaneous injection,

<sup>5</sup>Novocaine or procaine is the hydrochloride of p-aminobenzoylethylamine thanol.



novocaine solutions alkalized with sodium bicarbonate, produced greater toxicity. Gardner and Semb (9) express the belief that when a local anesthetic is injected into an organism, the pH of the solution will be adjusted by the buffer action of the body fluids; but that such an adjustment will not take place when injection is made into an isolated nerve. Their tests on ten local anesthetics injected into sciatic-gastrocnemius preparations of *Rana pipiens* showed that the bases alone were active.

It is difficult to harmonize the results of some of the previous investigations with those described here. In certain instances differences in organisms and techniques are apparently sufficient to account for the observed differences in toxic effect. Since organisms are often quite unlike in susceptibility to the same poison, it is also possible that there are differences among organisms with respect to susceptibility to molecules and ions of a given concentration of a single poisonous compound. Possibly the site of injection may alter the speed of toxic action; if injection is made into a region restricted by membranes, the molecules will diffuse rapidly across the membranes while diffusion of the ions will take place much more slowly; if the same compound is injected directly into the body cavity or into the blood stream, the molecules and ions may be quickly distributed throughout the body and fail to manifest a difference in toxic effect. Again the buffer systems of certain organisms may change rapidly the pH of such weak bases as novocaine and cocaine to a nearly uniform value within the body, whereas the pH of a stronger base like nicotine may not be altered so rapidly<sup>6</sup>. It seems that much more information must be available before these differences can be explained.

On the other hand, there is rather general agreement that alkaloids penetrate intact normal organisms as free bases rather than as ions (Höber, 11). This seems to be true of nicotine when speed of penetration into several species of insects is measured by survival time (20, 21), and when penetration into the goldfish *Carassius auratus* L. (8), is measured either by survival time or by the rate of accumulation of the alkaloid in the body.

#### SUMMARY

To determine whether molecular nicotine is more effective than ionic nicotine when injected into the American cockroach, *Periplaneta americana* (L.), relatively large doses and sublethal, paralytic doses of the alkaloid at several hydrogen ion concentrations were administered to adult insects. Injection was made into the body cavity by puncture at the base of a posterior leg, the method resembling intraperitoneal injection of vertebrate animals.

No significant difference was observed in the mortality from relatively large doses of injected nicotine that could be related to the pH of the solutions; nor did the paralytic and recovery times after injection of

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<sup>6</sup> See Pearson and Richardson (19) for a brief reference to this subject, based upon experiments with arsenious acid solutions administered *per os* to adult houseflies.

sublethal doses vary consistently, throughout the experiments, with the hydrogen ion concentration. Such differences as did occur did not support the hypothesis that injected nicotine molecules are more active than injected ions.

Male cockroaches are more susceptible than the females to large doses of injected nicotine; but no sex difference was observed in the reaction times or recovery times of insects injected with sublethal, paralytic doses.

#### ACKNOWLEDGEMENT

This investigation was supported by a grant from the Rockefeller Fluid Research Fund which is gratefully acknowledged by the authors.

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# NATURE OF EIMERIA NIESCHULZI-GROWTH-PROMOTING POTENCY OF FEEDING STUFFS

## I. POSITIVE EFFECT OF GRAY SHORTS

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Received February, 8, 1940

Becker and Morehouse (1937) suggested the name coccidibios for a "hypothetical coccidium stimulant," occurring in "many materials used as food for animals." The increased number of oocysts of *Eimeria nieschulzi* eliminated when certain feeding stuffs were included in the ration of the rat, and the resulting depression in numbers when these materials were either omitted or replaced with certain substitutes, suggested the existence of such a substance. The parasite-restricting effect exhibited by certain diets was tentatively explained by "the absence from the diet of a peculiar nutrient of the coccidium." The hypothesized material was declared to occur in connection with vitamins B and G, and to be thermostable. Obviously the hypothesis required further testing, and it was promised in a recent paper (Becker and Waters, 1939) that it would be made the central problem for future investigation.

A series of papers (Becker and Derbyshire, 1937, 1938; Becker and Waters, 1939) has brought out that feeding stuffs, tested in a basal ration, exhibit a wide spread of *Eimeria nieschulzi*-growth-promoting potency; for example, the potency of soy bean oil meal with 44.3 per cent protein fed at the 10 per cent level was measured by the factor 0.51, while wheat flour middlings (gray shorts) with 17.1 per cent protein and fed at the 30 per cent level was rated 2.65. It is of fundamental importance at the start of an investigation of this nature to clarify the two following considerations: Is the disparity in behavior exhibited by such materials as soy bean oil meal and gray shorts due to positive properties possessed by the oil meal or by the shorts? Does the oil meal supply a depressant, or do the shorts provide materials or conditions that encourage more abundant life for the protozoon in the rat's intestine?

The procedure outlined elsewhere (Becker and Derbyshire, 1937) was followed in general, but was modified when considered expedient. The life cycle of the parasite has been outlined in the paper cited, but in detail by Roudabush (1937). The infection culminates in the production of oocysts which are eliminated in the faeces of the host within a period of a few days after their appearance in the intestinal wall. The evolvment of the sexual forms from the last generation schizonts and the subsequent development of the oocysts terminates the infection (see Roudabush, 1937) and makes it a fairly safe presumption that oocyst counts are a reliable criterion of the extent of multiplication of the parasite in the host.



**EXPERIMENT 1.** Ration 1 ( $R_1$ ) and Ration 2 ( $R_2$ ) (see table 1) were fed to separate groups of young rats for 9 days. At the end of the ninth day and on the twelfth day each rat was inoculated directly into the stomach with 2,000 sporulated oocysts of *Eimeria nieschulzi*. Collections of the oocysts were made over a period of eight days, commencing early the morning of the seventh day. Oocyst counts were made by the dilution method. The rats were weighed individually on the day they were started on the test rations, and again on the ninth and sixteenth days.

TABLE 1. *Composition of rations used in Experiments 1 and 2*

| Feeding stuff            | Ration 1<br>( $R_1$ ) | Ration 2<br>( $R_2$ ) | Ration 3<br>( $R_3$ ) | Ration 4<br>( $R_4$ ) | Ration 5<br>( $R_5$ ) | Ration 6<br>( $R_6$ ) |
|--------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Beet sugar               | 36                    | 46                    | 41                    | 44                    | 71                    | 42                    |
| Casein<br>(commercial)   | 15                    | 15                    | 10                    | 17                    | 10                    | 9                     |
| Normal salts<br>(Harris) | 4                     | 4                     | 4                     | 4                     | 4                     | 4                     |
| Lard                     | 3                     | 3                     | 3                     | 3                     | 3                     | 3                     |
| Cod liver oil            | 2                     | 2                     | 2                     | 2                     | 2                     | 2                     |
| Gray shorts              | 30                    | 30                    | 30                    | 30                    | —                     | 30                    |
| Soy bean oil<br>meal     | 10                    | —                     | 10                    | —                     | 10                    | 10                    |

As shown in table 2, two trials with Rations  $R_1$  and  $R_2$  showed that the former, which contained 10 parts of oil meal and 30 parts of shorts, did not condition the host for the development of a significantly lesser number of oocysts than the latter, which contained 30 parts of shorts.  $R_3$  and  $R_4$  (see table 1) were planned so that the total protein content would be approximately the same. Two tests with these rations likewise showed clearly that the presence of the soy bean in the ration did not inhibit parasite development.

**EXPERIMENT 2.** Two rations,  $R_5$  with 10 parts soy bean oil meal and  $R_6$  with 10 parts of the oil meal and 30 parts gray shorts, were tested simultaneously for their respective coccidium-growth-promoting potencies. In two trials  $R_6$  showed better than 3:1 superiority, constituting proof that the wheat product in some manner or other conditioned the host for better development of the final stages of the parasite than did the soy bean oil meal.

Since the shorts contained decidedly more of the positive factor, the adoption of  $R_5$  as the control or reference ration was indicated. The lot of shorts used in this test was used for extracts.

**EXPERIMENT 3.** Ether, 96 per cent alcohol, petroleum ether, and water were the solvents used for extracting gray shorts. The test ration in each case consisted of the control ration supplemented with extract equivalent to 30 grams of shorts for each 100 grams of the ration. The

TABLE 2: Mean weight gains and oocyst counts for lots of rats on test rations

| Experi-<br>ment | Trial | Ration                       | No.<br>Rats | Mean<br>initial Wt.<br>(grams) | 16-day<br>Wt. gain<br>(grams) | Mean<br>number oocysts<br>(millions) |
|-----------------|-------|------------------------------|-------------|--------------------------------|-------------------------------|--------------------------------------|
| 1               | 1     | R <sub>1</sub>               | 4           | 70                             | 59                            | 119.0                                |
|                 |       | R <sub>2</sub>               | 4           | 70                             | 53                            | 155.0                                |
|                 | 2     | R <sub>1</sub>               | 4           | 69                             | 56                            | 190.2                                |
|                 |       | R <sub>2</sub>               | 4           | 69                             | 67                            | 182.5                                |
|                 | 3     | R <sub>3</sub>               | 5           | 72                             | 70                            | 112.0                                |
|                 |       | R <sub>4</sub>               | 5           | 73                             | 73                            | 103.8                                |
|                 | 4     | R <sub>3</sub>               | 7           | 69                             | 71                            | 129.6                                |
|                 |       | R <sub>4</sub>               | 7           | 69                             | 72                            | 104.4                                |
| 2               | 1     | R <sub>5</sub>               | 5           | 74                             | 25                            | 146.4                                |
|                 |       | R <sub>6</sub>               | 5           | 74                             | 64                            | 451.2                                |
|                 | 2     | R <sub>5</sub>               | 12          | 70                             | 23                            | 105.5                                |
|                 |       | R <sub>6</sub>               | 12          | 69                             | 55                            | 373.3                                |
| 3               | 1     | R <sub>5</sub> +EE*          | 4           | 61                             | 16                            | 121.0                                |
|                 |       | R <sub>5</sub>               | 3           | 68                             | 11                            | 220.7                                |
|                 | 2     | R <sub>5</sub> +EE*          | 3           | 59                             | 18                            | 135.2                                |
|                 |       | R <sub>5</sub>               | 2           | 68                             | 24                            | 220.0                                |
|                 | 3     | R <sub>5</sub> +AE*          | 3           | 54                             | 46                            | 164.0                                |
|                 |       | R <sub>5</sub>               | 3           | 56                             | 24                            | 201.0                                |
|                 | 4     | R <sub>5</sub> +AE*          | 3           | 72                             | 39                            | 77.7                                 |
|                 |       | R <sub>5</sub>               | 3           | 74                             | 20                            | 156.3                                |
|                 | 5     | R <sub>5</sub> +AE*          | 3           | 74                             | 55                            | 164.0                                |
|                 |       | R <sub>5</sub>               | 3           | 74                             | 37                            | 201.0                                |
|                 | 6     | R <sub>5</sub> +PE*          | 3           | 72                             | 23                            | 101.7                                |
|                 |       | R <sub>5</sub>               | 3           | 73                             | 17                            | 119.0                                |
|                 | 7     | R <sub>5</sub> +PE*          | 5           | 64                             | 17                            | 64.0                                 |
|                 |       | R <sub>5</sub>               | 5           | 65                             | 16                            | 54.0                                 |
|                 | 8     | R <sub>5</sub> +WE*          | 6           | 71                             | 36                            | 152.0                                |
|                 |       | R <sub>5</sub>               | 6           | 70                             | 19                            | 160.0                                |
|                 | 9     | R <sub>5</sub> +AWE*         | 3           | 62                             | 27                            | 48.0                                 |
|                 |       | R <sub>5</sub>               | 3           | 59                             | 21                            | 24.0                                 |
|                 | 10    | R <sub>5</sub> +AWE*         | 7           | 70                             | 30                            | 206.0                                |
|                 |       | R <sub>5</sub>               | 8           | 72                             | 20                            | 111.0                                |
|                 | 11    | R <sub>5</sub> +AE+<br>EE+PE | 4           | 74                             | —                             | 86.8                                 |
|                 |       | R <sub>5</sub>               | 4           | 73                             | —                             | 146.8                                |

\*Note: E.E.—Ether extract of gray shorts.

A.E.—96% alcohol extract of gray shorts.

P.E.—Petroleum ether extract of gray shorts.

W.E.—Aqueous extract of gray shorts.

A.W.E.—Autoclaved water extract of gray shorts.

extracts were reduced in volume under reduced pressure, and dried onto the mixture of sugar, salts, casein, and oil meal in an oven at a temperature of 55°C. Lard and codliver oil were added just before the rations were fed. The size of the infecting doses was not the same in all experiments, a fact which accounts in part for the variation in counts. Another factor was the age of the culture.

A glance at table 2 shows that oocyst production was not promoted by ether extract, alcohol extract, and petroleum ether extracts. In fact, the counts for the rations supplemented with ether and 96 per cent alcohol extract were decidedly lower than for the control ration, and depression of oocyst production is indicated. Perhaps the explanation is to be found in the thiamin chloride content of the extracts (Becker, 1939). Likewise, a supplement consisting of a mixture of the 3 extracts, each with the same shorts equivalent as before, depressed oocyst counts (Trial 11).

The aqueous extracts were difficult to prepare on account of difficulty in clearing them of turbidity. Extract WE was prepared by boiling a weighed amount of shorts with a liberal amount of water for half an hour, and filtering out as much as possible of the solid material with six layers of cheese cloth. After repeated high-speed centrifuging a solution with very little turbidity was obtained. When this was concentrated under reduced pressure a flocculent material separated out. The clear fluid was drawn off and used for the extract.

Since in one experiment increased rat growth was attained with extract WE, but not increased oocyst production, another similar extract A.W.E. was prepared, except that in this case the shorts mixed with hot water to form a thin gruel were autoclaved for 4.5 hours at 120°C. before the separation was made. Two tests showed almost twice as much coccidium development in the rats that received this extract as in the controls on R<sub>5</sub>.

#### SUMMARY

(1) The disparity between the number of oocysts of *Eimeria nieschulzi* eliminated by rats on basal ration supplemented with soy bean oil meal and on basal ration supplemented with wheat shorts of comparable protein content is probably to be attributed to the positive coccidium-growth-promoting potency of the middlings rather than to positive suppressive action of the soy bean oil meal.

(2) Ether, alcohol, and petroleum ether extracts of gray shorts did not promote the development of the protozoon.

(3) Autoclaved mixtures of shorts and water yielded an extract that exhibited coccidium-growth-promoting potency.

(4) The substance (or substances) favoring the development of the parasite when added to the particular reference ration (R<sub>5</sub>) used is water-soluble and heat-stable.

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## ACKNOWLEDGEMENT

The investigation was supported in part by grants from the Industrial Science Research Institute at Iowa State College and the American Academy of Arts and Sciences. The author is grateful to Mr. P. C. Waters and Mr. R. I. Dilworth for technical assistance.





## SOME NEW AND HERETOFORE UNRECORDED NABIDAE (HEMIPTERA)

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The following notes and descriptions are in continuation of a series of contributions dealing with the Nabidae. My indebtedness to the authorities of the British Museum of Natural History for the loan of specimens is gratefully acknowledged.

### *Pachynomus africanus*, n. sp.

Dark brown, the vertex, pronotum, and apical marking of femora sometimes brownish black; the apical part of head, the collum, rostrum, antennae, tibiae and tarsi testaceous to fuscotestaceous; the coxae, trochanters, basal half of front femora, and basal two-thirds of middle and hind femora pale to golden testaceous. Somewhat dull and opaque; the legs and antennae with extremely fine, sparse pubescence. Pronotum, scutellum, hemelytra, and body beneath coarsely punctate throughout. Head, measured to collum, longer than broad (28:23); vertex strongly arched, narrow (9), punctate down the middle and at base; gula wide, somewhat swollen, with a short, seta-like spine ventrad to base of antenniferous tubercle. Eyes fairly large, elongate-oval as seen from above; proportions, length:width:depth = 12:7:13. Antennae slender, segment I stout, II and III subequal in thickness; proportions, 9:29:25:29:34. Rostrum stout, segments II and III subequal, each one-half longer than IV.

Pronotum broader than long (54:32), somewhat flat and not especially arched; the lateral edge distinctly margined and feebly convex, not sinuate; the disc irregularly, rugosely punctate throughout; the hind margin, broadly, shallowly, emarginate. Scutellum large, rugose. Hemelytra not covering abdomen, strongly coriaceous and roughly sculptured to granular, the veins raised and prominent; membrane fuscous brown, just attaining apex of abdomen, with a single vein arising from the outer of the two closed cells. Anterior femora very stout, only about twice as long as greatest diameter (50:25), the inner edge almost straight and armed with a multiple row of short, piceous peg-like teeth and a row of long, seta-like spines. Anterior trochanters large, with several stout teeth in front. Anterior tibiae bowed, dentate within, and provided with a small apical pad. Intermediate femora only moderately swollen, armed on inner surface both in front and behind with teeth and spines, their tibiae strongly setose within. Meso-sternum swollen. Metapleuron as broad as long, strongly transversely rugose, without ostiole. Abdomen widened, the connexiva exposed from above. Venter strongly rugulose, segments two to six each with an extremely long fine hair arising from a papilla near its middle,

those on each side placed in an oblique line which runs outward to apex of connexivum.

*Length*: 8.18 mm. *Width*: pronotum, 2.27 mm.; abdomen, 3.13 mm.

*Holotype*: N. Nigeria, S. E. Kano, Azare, 1925, Dr. L. Lloyd (Br. Museum). *Paratype*: three specimens (females?) taken with type (British Museum and author's collection).

This species belongs to the subgenus *Punctius* Stål. In general color and in markings of the legs as well as in many other ways it resembles *Pachynomus* (*Camarochilus*) *americanus* Harris and *P. confusus* Harris. Apparently the specimens in the type series are all females; however, due to the short series it has not been felt advisable to sacrifice a specimen for dissection to make certain of this point.

### *Prostemma amyoti* Reuter

1909 *Prostemma amyoti* Reuter, Acta Soc. Sci. Fenn., 37 (2): 17.

A macropterous female from Senegal, and belonging to the Deutsche Entomologische Museum, was determined by me as this species in 1935. There is now at hand a macropterous female, from N. Nigeria, Azare, 1925, Dr. L. L. Lloyd (British Museum collection), that is tentatively referred to *amyoti*. Apparently the species is closely related to *Prostemma breddini* Poppius and *Prostemma falckensteini* Stein and it will be necessary to have specimens in series and especially males to establish the specific limits of the three forms.

### *Prostemma belidis*, n. sp.

Oblong, slender, narrowing somewhat anteriorly; strongly setose; shiny, the hemelytra and scutellum duller; pronotum and abdomen strongly punctate. Fuscous black to piceous, the anterior lobe of pronotum rufo-piceous, the posterior lobe and scutellum red. Clavus testaceous; the basal part of embolium, a narrow streak on corium next clavus, a transverse fascia entirely across corium before its apex, a small triangle in outer basal angle of membrane and a large spot at apex of membrane white. Antennae testaceous, the distal segments fuscotestaceous. Legs piceous, all trochanters, the basal third of anterior femora and basal half of middle and hind femora, and apices of all femora pale testaceous. Thorax beneath rufous, the venter piceous. Head faintly broader than long (22:20), the vertex wide (8), smooth, shiny. Eyes dark, variegated; proportions, 10:8:12. Antennae clothed with short, semi-erect hairs, the distal segments also with fine pubescence; segment I surpassing apex of head; proportions 6:4:17:17:16. Rostrum piceous, stout, reaching between front coxae; proportions, 12:15:5.

Pronotum subequally as long as broad (35:36), the posterior lobe and the sides and front of anterior lobe thickly, coarsely punctate; the smooth disc of front lobe longitudinally sulcate in the middle; the sides feebly margined. Scutellum punctate, the apex shiny. Hemelytra reduced, strongly narrowed backwards from the embolial fracture (at about basal

segment of abdomen), the membrane long and very narrow, slightly longer than corium (35:30) and only slightly overlapping. Abdomen strongly pilose and setose above, the connexivum semi-vertical. All femora stout, setose, unarmed, the anterior ones only about twice as long (measured within) as deep (24:11). Front tibia testaceous, stout, serrately denticulate within, the apical pad fairly long (8); coxae piceous. Ostiolar channel shiny, wide, somewhat triangular. Venter thickly pubescent and setose.

*Length*: 6.52 mm. *Width*: pronotum, 1.52 mm.; abdomen, 2.0 mm.

*Holotype*: female, Domira Bay, Nyasaland, Africa, 1670 ft., March 10, 1915, Dr. W. A. Lanborn (British Museum). *Paratypes*: four females taken with the type (in collection of Br. Mus. and of author).

This species belongs to the subgenus *Scelotrichia* Reuter.

*Phorticus abdominalis*, n. sp.

(Plate I)

Small, obovate; rather thickly clothed on legs and body, except for median area of abdomen, with pale, semi-recumbent pubescence. Ochraceous to pale-brown, the posterior lobe of pronotum except for wide median spot, the abdomen above except base and wide median longitudinal fascia that tapers to middle of last segment, and the venter dark brown to fuscous black. Head somewhat embrowned around eyes and at base of vertex. Scutellum embrowned at the sides basally. Antennae testaceous, the incisures, the basal half of segment II and the base and apex of I paler. Legs pale testaceous, a band at the distal third of the front femora (broadly widened on posterior surface), the distal third of middle and hind femora, and the base of all tibiae fuscous brown.

Head as long as broad (11). Vertex arched, wide (5). Eye oval, reddish; proportions, 5:3:6. Antennae pilose, segment I surpassing apex of head; proportions, 5:9:9:9. Rostrum long, the second segment one-third longer than second antennal segment. Pronotum faintly broader than long (21:19), evenly arched and imperceptibly divided into lobes; sides faintly constricted before apex, the disc indistinctly sulcate at the middle. Scutellum distinctly broader than long, raised, the base impressed, bifoveate. Hemelytra white, greatly reduced, extending only to middle of first abdominal segment, the apex truncate and oblique, inner apical angle not attaining apex of scutellum. Abdomen shiny, tumid, apparently impunctate except in the incisures, the lateral edges broadly arched. Anterior femora strongly widened and dentate at the middle, then denticulate to the apex. Anterior tibiae stout, armed within.

*Length*: 3.44 mm. *Width*: pronotum, 0.91 mm.; abdomen 1.34 mm.

*Holotype*, apterous female, India: Namsoo, Darjeeling, 2100 ft., XI-20, H. Stevens. (British Museum.)

The apical fifth of pronotum and the median spot of the posterior lobe are noticeably paler than the anterior lobe; and the apices of the tibiae and tarsi are slightly darkened. Venter paler at base and in the middle.



*Phorticus socialis*, n. sp.

Oblong, rather thickly clothed with short, pale pile. Buff, the head deep blackish brown, shiny; scutellum brown; membrane smoky brown, paler at margins; the cell of clavus and the two inner cells of corium whitish; legs, rostrum and antennae pale testaceous to buff. Head short, broader than long (13:11); vertex swollen, broad (5). Eyes dark, distinctly pilose, short, the proportions 6:4:8. Ocelli large. Antennae fairly long, darkened distally, segment I surpassing apex of head by nearly half of its own length; proportion of segments, 7:16:13:13. Rostrum long, extending between hind coxae, segment III stout; proportions, 14:10:5. Pronotum much broader than long (32:26), rather uniformly arched above, the sides sinuate, the transverse impression punctate, the disc of anterior lobe finely sulcate down the middle, the hind lobe slightly paler than front lobe, with an indistinct, fine carina at middle behind transverse impression. Scutellum slightly broader than long; irregularly punctate on the impressed basal portion, the basal foveae deep and wide apart; the lateral edges slightly sinuate, each with a row of six or seven large, fovea-like punctures. Hemelytra distinctly pilose, the veins stout, the inner ones paralleled by punctures basally; membrane large, faintly exceeding apex of abdomen, its veins stout. Connexivum shiny, semivertical, not covered by hemelytra. Anterior femora, measured below, more than twice as long as depth measured to base of large spine (20:8), armed within on distal half with a single, irregular row of minute piceous denticles, the median angulation stout. Front tibiae strongly widened distally, the inner margin curved and serrate, the apical pad nearly as long as diameter of femur (7:8). Mesosternum and metasternum strongly carinate, slightly darker than venter. Ostiolar canal broad, shiny, curved posteriorly. Propleuron produced downward into a short angulation at the hind edge of the acetabulum. Venter strongly pilose, the incisures punctate, the genital valves slightly darkened.

*Length*: 4.52 mm. *Width*: pronotum, 1.39 mm.; abdomen, 1.60 mm.

*Holotype*: Macropterous female, Brisbane, Australia, Nov. 27, 1930, H. Hacker, taken at light (author's collection).

The species is unique in its more or less uniform color of pronotum, body and appendages, with contrastingly dark head, scutellum and membrane and pale cells of clavus and corium.

*Phorticus varicolor* Distant

(Plate II)

1919 *Phorticus varicolor* Distant, Ann. Mag. Nat. Hist., (9) 4: 79.

Two specimens, Chikkaballapura, S. India, T. V. Campbell. These are topotypical and represent the heretofore unknown brachypterous form.

*Brachypterous form*: Head and abdomen piceous, the latter paler basally. Pronotum reddish ochraceous, the humeri fuscous. Antennae testaceous, II darkest. Rostrum testaceous. Legs flavo-testaceous, the

apices of tibia faintly darkened. Hemelytra pale, the apex fuscous. Antennal proportions, 4:10:9:11. Rostrum, 11:10:6. Pronotum only faintly broader than long (26:25), rather prominently clothed with short, almost erect, dark, stiff hairs; the sides broadly rounded in front, only slightly constricted behind the middle; the basal lobe faintly carinate down the middle. Hemelytra reaching the middle of first abdominal segment, not contiguous behind scutellum, the apex truncate and only slightly oblique. Abdomen thickly pilose, and strongly, coarsely punctate; the connexiva semivertical. Legs stout, thickly pilose and setose; the anterior femora, measured below, more than twice as long as deep (18:7), with a short, broad tooth at the middle and minute denticulations from there to apex. Anterior femora short, stout, the inner surface curved and serrately denticulate; the apical pad equal to length of an eye (5).

*Length*: 3.7 mm. *Width*: pronotum, 1.13 mm.; abdomen, 1.35 mm.

*Morphotype* and *paramorphotype*, males, topotypical (Br. Museum and author's collection).

*Phorticus flavus* var. *breviatus*, n. var.

Oblong, rather densely clothed with pale pubescence, also somewhat setose. Head shiny, piceous, the apex paler, as long as broad (19). Eyes dark; proportions, 8:5:10. Pronotum broader than long (50:45), strongly rounded in front and prominently constricted behind the middle, distinctly irregularly punctate in the transverse impression, the sides of the front lobe, and the posterior lobe except at median area fuscous brown; the remainder ochraceous, paler in front and at middle of posterior lobe. Scutellum fuscous, impressed basally as in other forms, the apex rather broad and somewhat bifid. Hemelytra fuscous brown, darkened distally, the basal third of corium and basal half of clavus ochraceous; the inner vein of corium and the claval veins stout, paralleled by coarse punctures; membrane smoky black, attaining apex of abdomen. Basal antennal segment testaceous, segment II darker, stout, its length subequal to width of head through eyes. Rostrum pale, extending on mesosternum, segment III swollen; proportions, 19:13:7. Sides of thorax embrowned to fuscous, paler in front. Mesosternum strongly carinate. Venter pale testaceous, coarsely punctate at the incisures. Legs pale, stout; the front femora evenly arched above and below, as measured along lower surface only twice as long as thick (28:14), armed at the middle with a very large tooth; front tibia curved, armed within, the apical pad in length equal to one-half of diameter of femur.

*Length*: 6.3 mm. *Width*: pronotum, 2.17 mm.; abdomen, 2.43 mm.

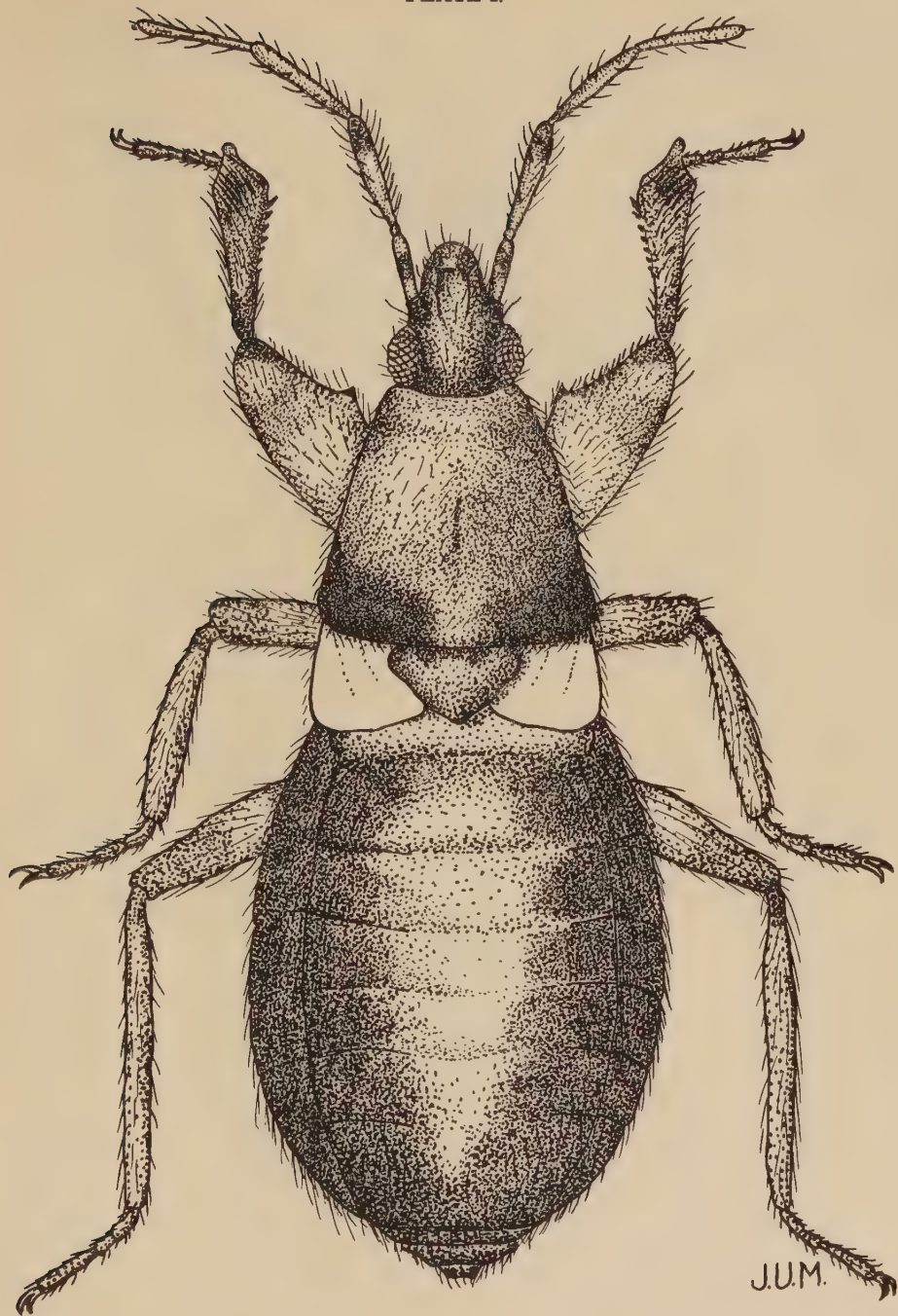
*Holotype*: female, Salisbury, South Africa, 1897 (British Museum).

The tooth on front femur is serrate in front and the femur is armed from it to apex with two rows of minute denticulations.

## PLATE I.

*Phorticus abdominalis*, n. sp.

PLATE I.



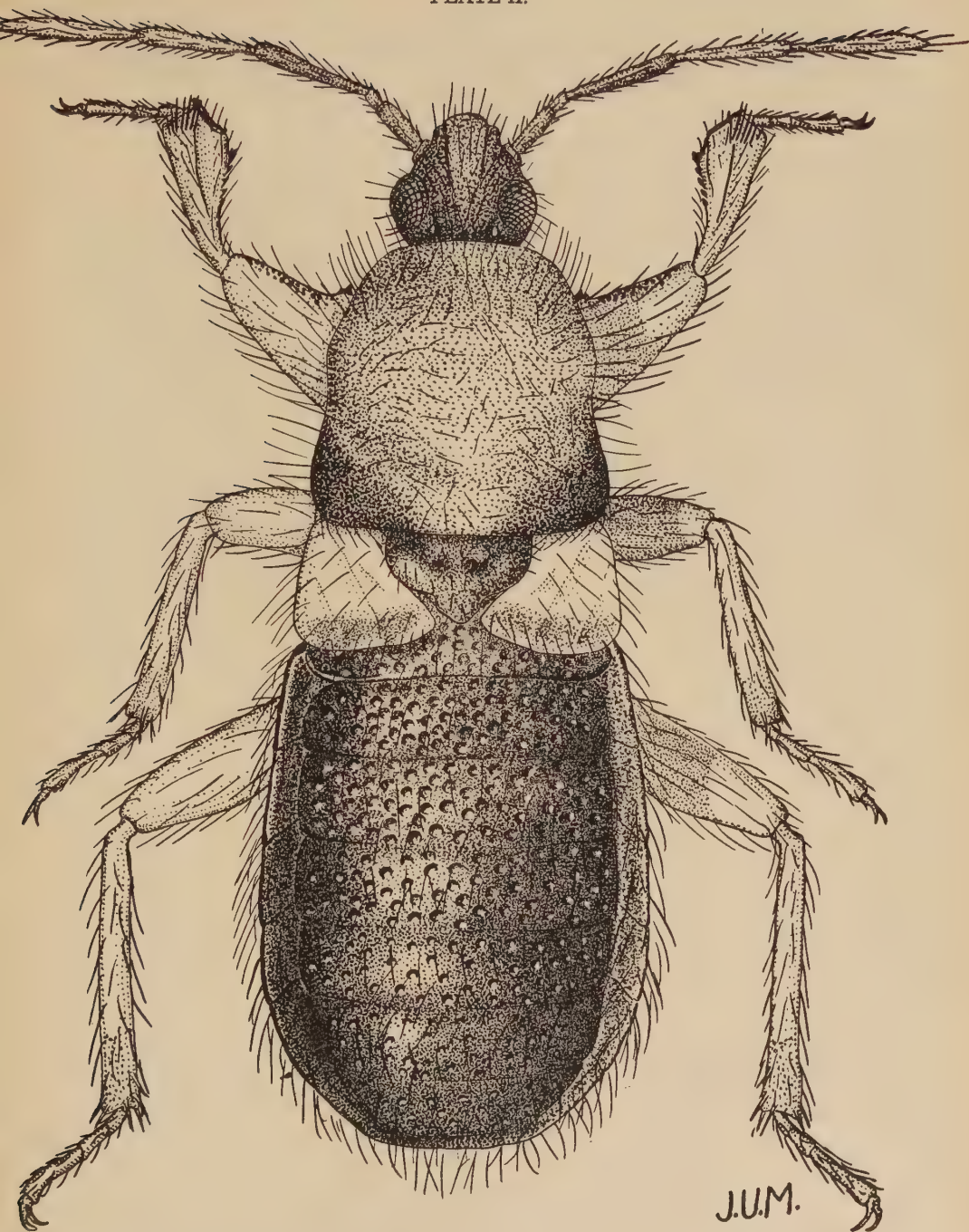
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## PLATE II.

*Phorticus varicolor* Distant.

PLATE II.





# A FUNCTIONAL METHOD FOR THE SOLUTION OF THIN PLATE PROBLEMS APPLIED TO A SQUARE, CLAMPED PLATE WITH A CENTRAL POINT LOAD

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The present paper employs a new point of view for obtaining approximate solutions of linear differential equations. In this method the approximations are determined by the use of functionals. A person experienced in the approximate solution of such differential equations will recognize that the results obtained by the use of functionals are, at times, the equivalent of results obtained by the use of other approximation methods. As a matter of fact, it will be found that the functional method welds most of the standard methods, and many more, into a coherent whole.

While the particular problem to which we shall apply this method has been solved using other points of view, the present solution illustrates the versatility of the functional idea. In particular, this work illustrates how solutions are obtained by this method when no functions which satisfy either the boundary conditions or the differential equations are known.

The general method as first enunciated by Gross<sup>1,2</sup> is as follows: We desire a solution to the linear operational equation

$$L(w) = f \tag{1}$$

and the  $b$  linear boundary conditions

$$B_l(w) = g \text{ when } \lambda_l = 0 \tag{2}$$

$$(l = 1, 2, \dots, b).$$

in which

$$\lambda_l \equiv \lambda_l(x_1, x_2, \dots, x_n) = 0 \tag{4}$$

are the equations of the boundary.

It is assumed that  $w$  can be represented by a sum of expansion functions,

$$w = a_i \phi_i, \tag{5}$$

where the  $a_i$ 's are constants to be determined and the  $\phi_i$ 's are known functions. A subscript, repeated in a term, has the usual meaning that the term is to be summed for all values of the subscript, *e. g.*

$$a_i \phi_i = \sum_{i=0}^{\infty} a_i \phi_i$$

unless the contrary is expressly stated. If the  $\phi_i$ 's constitute a complete



set of functions, it is known that a very wide range of functions can be expressed by such a sum. It is assumed that a finite number of them will be an approximation  $w^k$  to the solution  $w$ .

$$w \cong w^k = a_m^k \phi_m (m = 0, 1, 2, \dots, k) \quad (7)$$

Substitution of the finite expansion (7) for  $w$  in equations (1) and (2) gives

$$L(a_m^k \phi_m) \cong f$$

or

$$a_m^k L(\phi_m) - f \cong 0 \quad (8)$$

and

$$a_m^k B_l(\phi_m) \cong g \text{ on the boundary} \quad (9)$$

since the linear operators commute the  $a_m^k$ 's.

Suppose we now choose two functional families  $F_j$  and  $G_j$  which are linear. The  $F$ 's are defined in the region  $S$  of the plate; the  $G$ 's on  $s$ , the boundary of the plate. The approximation defined by the functional families  $F_j$  and  $G_j$  and the functions of expansion,  $\phi_m$  is determined when we require that the coefficients,  $a_m^k$  satisfy the equations:

$$F_j[a_m^k L(\phi_m) - f] = 0 (j = 0, 1, \dots, k-r); \quad (10)$$

$$G_j[a_m^k B_l(\phi_m) - g] = 0 (j = k-r, \dots, k). \quad (11)$$

We have thus reduced the problem of finding an approximate solution of the differential equation to the problem of finding a solution to an arbitrary number of linear equations in the  $a_m^k$ 's. The number of equations to be solved is arbitrary because it depends upon the number of terms in the assumed approximation function. The resultant linear equations in the  $a_m^k$ 's are then solved for the  $a_m^k$ 's and an approximation for  $w$  results.

Thus a given set of functionals and functions define an approximation to the solution of the problem. The properties of these approximations, such as their limits of error, have not been entirely determined<sup>2</sup>, partly because of the complete generality of the method.

The choice of the equations for the determination of the  $a_m^k$ 's is usually guided by the relative degrees of approximation desired for the differential equation (1) and the boundary conditions (2). In general, different choices of equations for the determination of the  $a_m^k$ 's leads to different approximations to  $w$ . For an approximation with more terms, equations (10) and (11) with more terms in  $a_m^k \phi_m$  and hence more, and usually different equations in the  $a_m^k$ 's are to be solved. In general, the resultant  $a_m^k$ 's will be different from different approximations. If the  $a_m^k$ 's remain the same such approximations may be called stable, e.g. Fourier series.

It is easily seen that most of the standard methods for determining approximate solutions are particular examples of this functional method.

If the boundary conditions are homogeneous we may be able to choose expansion functions so that each (and hence their sum) satisfies these conditions. Suppose we choose to satisfy the differential equation by application of the functional family

$$F_j [\ ] \equiv \int \phi_m [\ ] d\tau \quad (12)$$

to (8). There results

$$a_m^k \int \phi_m L(\phi_m) d\tau = \int \phi_m f d\tau \quad (13)$$

and we have the same equations as are given by the Ritz<sup>3</sup> method. Suppose we choose to satisfy the differential equation by employing the functional family

$$F_j [\ ] \equiv \int L(\phi_m) [\ ] d\tau. \quad (14)$$

There results

$$a_m^k \int [L(\phi_m)]^2 d\tau = \int f L(\phi_m) d\tau \quad (15)$$

which are the equations defining the Bousinesq<sup>4</sup> or "least square" approximation.

If our problem is of such a nature that  $w = f(s)$  on  $I$  and  $\frac{\partial w}{\partial \nu} = g(s)$  on  $II$ , we may be able to choose the expansion functions such that each satisfies the differential equation and their sum satisfies  $\frac{\partial w}{\partial \nu} = g(s)$  on  $II$ . If we apply the functional family

$$F_j \equiv \int_I \left[ \right] \frac{\partial \phi_j}{\partial \nu} ds \quad (16)$$

to the second boundary condition there results

$$\int_I \left[ a_m^k \phi_m - f(s) \right] \frac{\partial \phi_j}{\partial \nu} ds = 0 \quad (17)$$

These are the same equations as were determined by Trefftz<sup>5</sup>.

Gross<sup>1, 2</sup> has included many other well known mathematical methods and procedures as special cases of the functional method. This includes:

1. Perturbation theory.
2. Taylor's series expansion.
3. Expansion in orthogonal functions.
4. Certain solutions of integral equations.
5. Method of Krawtchouk.

## Homogeneous, Square, Clamped Plate with Center Point Load

*The Problem*

To solve this problem it is necessary to obtain a function  $w$ , representing the deflection of the plate due to the point load  $P$  at the origin of coordinates, which satisfies the differential equation

$$\nabla^4 w = \frac{P}{N} = 0, \quad \nabla^4 = \frac{\partial^4}{\partial x^4} + 2\frac{\partial^4}{\partial x^2 \partial y^2} + \frac{\partial^4}{\partial y^4}. \quad (18)$$

The coordinate plane is the plane of the middle surface of the undeflected plate with the origin at the centroid of the plate.

Because the plate is clamped,

$$\frac{\partial w}{\partial \nu} = 0 \quad \text{for} \quad \begin{matrix} x = \pm a \\ y = \pm a, \end{matrix} \quad (19)$$

$$w = 0 \quad \text{for} \quad \begin{matrix} x = \pm a \\ y = \pm a \end{matrix} \quad (20)$$

where  $a$  is half the distance between successive corners of the plate. Equation (19) is equivalent to the two equations

$$\begin{aligned} \frac{\partial w}{\partial x} &= 0 & \text{for} & \quad x = \pm a, \\ \frac{\partial w}{\partial y} &= 0 & \text{for} & \quad y = \pm a. \end{aligned} \quad (21)$$

Because  $\nabla^4 w = \frac{P}{N}$  we have the associated condition that the derivatives

must possess discontinuities of a type such that the shear integral has the value

$$\int \left[ N \frac{\partial \nabla^2 w}{\partial \nu} \right] d\theta = -P. \quad (22)$$

*Application of Theory*

For this problem we will use the simple functionals defined as follows:

$$F_k[f(x, y)] = f(x, y) \Big|_{\substack{x = x_k \\ y = y_k}} \quad (23)$$

This simply means that the function  $f(x, y)$  is reduced to its value at the point  $(x_k, y_k)$ , obtained by setting  $x = x_k$  and  $y = y_k$  in  $f(x, y)$ . The constants of the sum of expansion functions will be determined by evaluating

that solution at the same number of points as there are constants to be determined.

The expansion function chosen was

$$w^k = A_o(x, y) + R^k(x, y) \quad (24)$$

where  $R^k(x, y)$  is a polynomial in  $x$  and  $y$ , and  $A_o$  is a function satisfying the associated condition (22). The usual function for this purpose is

$$A_o(x, y) = \frac{Pr^2}{8\pi N} \log_e \frac{a}{r} \quad (25)$$

where  $r = \sqrt{x^2 + y^2}$  and  $N = E'I$ ,  $E' = \frac{E}{1 - \sigma^2}$  and  $I$  is the moment of in-

ertia of a unit cross section of area of the plate about the axis where it intersects the  $x, y$  plane. As usual  $E$  is Young's modulus and  $\sigma$  is Poisson's ratio. It is to be observed that the choice of expansion functions will greatly influence the rapidity of convergence of  $w^k$ .

Because of the symmetry conditions of the plate

$$R^k(x, y) = R^k(-x, y) = R^k(x, -y) = R^k(-x, -y). \quad (26)$$

This makes it necessary that we include only even powers of  $x$  and  $y$ . Also,

$$R^k(x, y) = R^k(y, x). \quad (27)$$

This places the further restriction upon  $R^k(x, y)$  that the coefficient of  $x^a y^b$  equal the coefficient of  $x^b y^a$ . With these conditions in mind we have chosen  $R^k$  a sum of homogeneous polynomials and

$$\begin{aligned} w^k = & \frac{P(x^2 + y^2)}{8\pi N} \log_e \frac{a}{r} + A_1 + A_2(x^2 + y^2) + A_3(x^4 + y^4) \\ & + A_4x^2y^2 + A_5(x^6 + y^6) + A_6(x^2y^4 + x^4y^2) \\ & + A_7x^4y^4 + A_8(x^2y^6 + x^6y^2) + A_9(x_8 + y_8) \\ & + A_{10}(x^{10} + y^{10}) + A_{11}(x^8y^2 + x^2y^8) + A_{12}(x^6y^4 + x^4y^6). \end{aligned} \quad (28)$$

If the functional (23) is now applied to the equations (18), (20) and (21), with  $w^k$  introduced, thus reducing  $x$  and  $y$  to their values at various points throughout the plane, we can solve the resultant equations for the coefficients in  $R^k(x, y)$ . We need only one of the slope equations (20) because of the symmetry of the problem. The points chosen are shown on the diagram of the plate (Figure 1); the resultant equations are equations (29) - (40).

The points represented by a dot are the points actually used and the points represented by a cross are those included by symmetry. The



points marked 3 were used in all three equations: normal derivative, displacement, and differential equation.

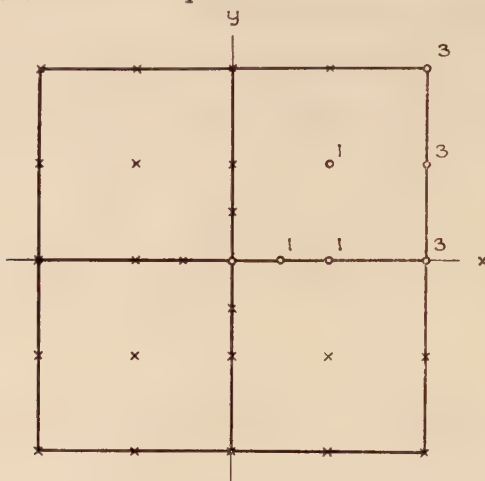


Figure 1

The boundary condition equations are:

$$x = a, y = 0, w = 0,$$

$$0 = A_1 + A_2 a^2 + A_3 a^4 + A_5 a^6 + A_9 a^8 + A_{20} a^{20}; \quad (29)$$

$$x = a, y = a/2, w = 0,$$

$$\begin{aligned} 0 = & \frac{{}^5/4 P a^2}{8\pi N} \log_e \frac{2}{\sqrt{5}} + A_1 + \frac{5}{4} A_2 a^2 + \frac{17}{16} A_3 a^4 \\ & + \frac{1}{4} A_4 a^4 + \frac{65}{64} A_5 a^6 + \frac{5}{16} A_6 a^6 + \frac{1}{16} A_7 a^8 + \frac{17}{64} A_8 a^8 \\ & + \frac{257}{256} A_9 a^8 + \frac{1025}{1024} A_{10} a^{10} + \frac{65}{256} A_{11} a^{10} + \frac{5}{64} A_{12} a^{10}; \end{aligned} \quad (30)$$

$$x = a, y = a, w = 0,$$

$$\begin{aligned} 0 = & \frac{2Pa^2}{8\pi N} \log_e \frac{1}{\sqrt{2}} + A_1 + 2A_2 a^2 + 2A_3 a^4 \\ & + A_4 a^4 + 2A_5 a^6 + 2A_6 a^6 + A_7 a^8 + 2A_8 a^8 \\ & + 2A_9 a^8 + 2A_{10} a^{10} + 2A_{11} a^{10} + 2A_{12} a^{10}; \end{aligned} \quad (31)$$

$$x = a, y = 0, \frac{\partial w}{\partial y} = 0,$$

$$0 = -\frac{Pa}{8\pi N} + 2A_2a + 4A_3a^3 + 6A_5a^5 + 8A_9a^7 + A_{10}a^{10}; \quad (32)$$

$$x = a, y = \frac{a}{2}, \frac{\partial w}{\partial x} = 0,$$

$$\begin{aligned} 0 = & \frac{Pa}{8\pi N} \left[ -1 + 2 \log_e \frac{2}{\sqrt{5}} \right] + 2A_2a + 4A_3a^3 + \frac{1}{2}A_4a^3 \\ & + 6A_5a^5 + \frac{9}{8}A_6a^5 + \frac{1}{4}A_7a^7 + \frac{49}{32}A_8a^7 + 8A_9a^7 \\ & + 10A_{10}a^9 + \frac{257}{128}A_{11}a^9 + \frac{7}{16}A_{12}a^9; \end{aligned} \quad (33)$$

$$x = a, y = a, \frac{\partial w}{\partial x} = 0,$$

$$\begin{aligned} 0 = & \frac{Pa}{8\pi N} \left[ -1 + 2 \log_e \frac{1}{\sqrt{2}} \right] + 2A_2a + 4A_3a^3 + 2A_4a^3 \\ & + 6A_5a^5 + 6A_6a^5 + 4A_7a^7 + 8A_8a^7 + 8A_9a^7 \\ & + 10A_{10}a^9 + 10A_{11}a^9 + 10A_{12}a^9; \end{aligned} \quad (34)$$

The differential equation conditions are:

$$x = a, y = 0, \nabla^4 w = 0,$$

$$\begin{aligned} 0 = & 8A_4 + 48A_3 + 360A_5a^2 + 72A_6a^2 + 24A_7a^4 \\ & + 120A_8a^4 + 224A_{11}a^6 + 1680A_9a^4 \\ & + 5040A_{10}a^6 + 24A_{12}a^6; \end{aligned} \quad (35)$$

$$x = a, y = a/2, \nabla^4 w = 0,$$

$$\begin{aligned} 0 = & 8A_4 + 48A_3 + 450A_5a^2 + 90A_6a^2 + \frac{195}{2}A_7a^4 \\ & + \frac{615}{2}A_8a^4 + \frac{1505}{2}A_{11}a^6 + 1785A_9a^4 \\ & + \frac{20475}{4}A_{10}a^6 + \frac{2895}{8}A_{12}a^6; \end{aligned} \quad (36)$$

$$x = a, y = a, \nabla^4 w = 0,$$

$$\begin{aligned} 0 = & 8A_4 + 48A_3 + 720A_5a^2 + 144A_6a^2 + 336A_7a^4 \\ & + 960A_8a^4 + 3808A_{11}a^6 + 3360A_9a^4 \\ & + 10080A_{10}a^6 + 2208A_{12}a^6; \end{aligned} \quad (37)$$

$$x = a/2, y = a/2, \nabla^4 w = 0,$$

$$\begin{aligned} 0 = & 8A_4 + 48A_3 + 180A_5a^2 + 36A_6a^2 + 21A_7a^4 \\ & + 60A_8a^4 + \frac{119}{2}A_{11}a^6 + 210A_9a^4 \\ & + \frac{315}{2}A_{10}a^6 + \frac{69}{2}A_{12}a^6; \end{aligned} \quad (38)$$

$$x = a/2, y = 0, \nabla^4 w = 0,$$

$$\begin{aligned} 0 = & 8A_4 + 48A_3 + 90A_5a^2 + 18A_6a^2 + \frac{3}{2}A_7a^4 \\ & + \frac{15}{2}A_8a^4 + \frac{7}{2}A_{11}a^6 + 105A_9a^4 \\ & + \frac{315}{4}A_{10}a^6 + \frac{3}{8}A_{12}a^6; \end{aligned} \quad (39)$$

$$x = a/4, y = 0, \nabla^4 w = 0,$$

$$\begin{aligned} 0 = & 8A_4 + 48A_3 + \frac{45}{2}A_5a^2 + \frac{9}{2}A_6a^2 + \frac{3}{32}A_7a^4 \\ & + \frac{15}{32}A_8a^4 + \frac{7}{128}A_{11}a^6 + \frac{105}{16}A_9a^4 \\ & + \frac{315}{256}A_{10}a^6 + \frac{3}{512}A_{12}a^6. \end{aligned} \quad (40)$$

### The Solution

The equations (29) - (40) are linear equations in the parameters  $A_1, A_2, A_3, \dots$ . These can be solved by the ordinary methods. The work of computation may be checked by substituting the calculated values of the parameters  $A_1, A_2, A_3, \dots$ , as derived. This shows that these constants are accurate to six places.

When these values are substituted in equation (28) there results the following approximation for  $w$ :

$$\begin{aligned}
\frac{8\pi N}{P} w^b = (x^2 + y^2) \log_e \frac{a}{r} - .562487a^2 + .556212(x^2 + y^2) \\
+ .075704 \frac{(x^4 + y^4)}{a^2} - .454207 \frac{x^2y^2}{a^2} \\
- .065438 \frac{(x^6 + y^6)}{a^4} + .327202 \frac{(x^2y^4 + x^4y^2)}{a^4} \\
+ .024818 \frac{x^4y^4}{a^6} - .009927 \frac{(x^2y^6 + x^6y^2)}{a^6} \\
+ .000352 \frac{(x^8 + y^8)}{a^8} - .002543 \frac{x^{10} + y^{10}}{a^8} \\
+ .068670 \frac{(x^8y^2 + x^2y^8)}{a^8} - .106820 \frac{x^6y^4 + x^4y^6}{a^8}.
\end{aligned}$$

The ratio of the maximum value of  $w^k$  on the boundary to the center deflection is .3%; of the normal derivative  $3/a\%$ . A homogeneous biharmonic polynomial has certain fixed ratios between its coefficients, hence, we can see how well the differential equation is satisfied by finding these ratios. These ratios, exact and as found from our solution are given in Table 1.

TABLE 1. Ratios of the coefficients of the biharmonic polynomials.

| Ratio           | Exact values | As found from Approximation function |
|-----------------|--------------|--------------------------------------|
| $A_1/A_3$       | — 6.00000    | — 5.99979                            |
| $A_6/A_5$       | — 5.00000    | — 5.00017                            |
| $A_8/A_6$       | — 28.0000    | — 28.2358                            |
| $A_7/A_9$       | 70.0000      | 70.5916                              |
| $A_{11}/A_{10}$ | — 27.0000    | — 27.0084                            |
| $A_{12}/A_{10}$ | 42.0000      | 42.0131                              |

Evaluation of the shear integral

$$8 \int_0^a \left[ N \frac{\partial \nabla^2 w}{\partial x} \right] dy = -P \quad (40)$$

around the boundary of the plate should give us  $-P$ . It actually gives

$$= (1.000027)P \quad (41)$$



The maximum deflection (the value of  $w$  at  $(0,0)$ ) from equation (39) is

$$.022452Pa^2/N \quad (42)$$

J. Barta<sup>6</sup> obtained by the Trefftz method

$$.0224Pa^2/N. \quad (43)$$

H. Marcus<sup>7</sup> obtained by difference equations

$$.02297Pa^2/N. \quad (44)$$

G. Pickett<sup>8</sup> obtained by energy minimization

$$.02153Pa^2/N. \quad (45)$$

The maximum bending moment is

$$- .125679P; \quad (46)$$

this is slightly dependent upon  $\sigma$  since  $w|_{y=a}$  is small in value but not zero. The moment was given above for  $\sigma = .3$ .

S. Timoshenko<sup>9</sup> obtained by superposition

$$- .1257P. \quad (47)$$

D. Young<sup>10</sup> obtained by a similar method

$$- .1257P. \quad (48)$$

G. Pickett obtained by energy minimization

$$- .1363P. \quad (49)$$

#### REFERENCES

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## PRELIMINARY STUDIES ON THE USE OF DINITRO-O-CRESOL DUSTS IN GRASSHOPPER CONTROL<sup>1</sup>

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Received May 17, 1940

The experimental use of proprietary compounds as contact insecticides to destroy mass-formations of locust and "tucuras" (grasshoppers) in the Republic of Argentina aroused the interest of the authors and suggested the desirability of testing certain nitro-cresols and related compounds on grasshoppers in Iowa.

Chemical analyses<sup>2</sup> of the two dusts most widely used in Argentina were as follows:

"A-Dust"—Active material 14 per cent, consisting entirely of 3,5-dinitro-o-cresol; inactive material 86 per cent, diatomaceous earth.

"B-Dust"—Active material 12 per cent, a mixture of 3,5-dinitro-o-cresol, some 5-nitro-o-cresol and sodium salts of these two compounds; inactive material 88 per cent, consisting of diatomaceous earth.

The use of dinitro-o-cresol compounds as insecticides is not new. As early as 1893 Lodeman (1893) imported antinonnin, a potassium dinitrocresylate compound, from Germany and reported that as a spray it was highly caustic on some fruit trees and that it did not control plant lice.

A rather extensive investigation on the relative toxicity of a long series of nitro-phenols and related compounds reported by Tattersfield, Gimingham and Morris (1925), stimulated interest in this field. This paper was followed by other papers from the same and other workers who have studied the use of dinitro compounds as contact sprays and particularly as ovicides. Kagy and Richardson (1936) published a brief review of the older literature in their paper which introduced the new compound, 2,4-dinitro-6-cyclohexylphenol, now receiving considerable study in several states.

About 1935, German entomologists began to experiment with dinitro-o-cresol and related compounds as contact dusts. Marcus (1937), Thiem (1938), Schwerdtfeler (1939) and Hofman (1940) have made important contributions on the subject and all cite the works of their contemporaries.

Twenty-five chemical compounds of technical grade or better obtained from reliable chemical houses were used in the preliminary experiments. In these tests, 50 adults of *Melanoplus bivittatus* Say (25 males

<sup>1</sup> Journal Paper No. J-753 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 649.

<sup>2</sup> The writers are greatly indebted to Mr. A. Goytea, Sub-Director Defensa Agrícola, Ministerio de Agricultura, Buenos Aires, for kindly supplying the authors with a supply of the two dust chemicals.



and 25 females) were individually dipped in finely ground dust of the various chemicals and then placed in screened cages for observation. The average time elapsing between dusting and death for each of the compounds is given in table 1.

The four most toxic compounds used in the preliminary series were then subjected to further tests. In these experiments, dust mixtures con-

TABLE 1. Average time elapsing between treatment and death of *Melanoplus bivittatus* adults when thoroughly coated with dust of various chemical compounds

| Chemical compound<br>(dust)           | Time required to kill (minutes) |       |              |       |
|---------------------------------------|---------------------------------|-------|--------------|-------|
|                                       | Males                           |       | Females      |       |
|                                       | Range                           | Mean* | Range        | Mean* |
| 3,5-Dinitro-o-cresol CP.              | 8 - 20                          | 14    | 14 - 29      | 21    |
| 3,5-Dinitro-o-cresol T.               | 9 - 18                          | 15    | 16 - 30      | 22    |
| 2,4-Dinitro phenol                    | 8 - 19                          | 14    | 17 - 34      | 22    |
| Sodium arsenite                       | 14 - 43                         | 32    | 18 - 54      | 35    |
| 2,4-Dinitro-o-cyclohexyl phenol       | 22 - 42                         | 33    | 52 - 97      | 71    |
| 3,5-Dinitro-o-cresol, sodium salt     | 28 - 76                         | 49    | 54 - 121     | 71    |
| 2,6-Dichloronitrophenol               | 48 - 73                         | 59    | 55 - 116     | 81    |
| 2,5-Dichloronitrobenzene              | 46 - 316                        | 182   | 84 - 253     | 168   |
| Hexochlorphenol                       | 125 - 156                       | 135   | 83 - 341     | 200   |
| O-Nitroaniline                        | 50 - 155                        | 103   | 56 - 447     | 202   |
| Sodium<br>dinitro-6-cyclohexylphenate | 69 - 316                        | 135   | 89 - 393     | 210   |
| 2,4-Dinitroanisole                    | 162 - 383                       | 288   | 261 - 413    | 330   |
| P-Nitrochlorobenzene                  | 150 - 233                       | 185   | 143 - 690    | 358   |
| P-Nitrophenetole                      | 154 - 289                       | 149   | 187 - 637    | 366   |
| P-Nitrotoluene                        | 68 - 454                        | 310   | 304 - 731    | 440   |
| M-Dinitrobenzene                      | 138 - 483                       | 272   | 380 - 1440+  | 510   |
| 2,4-Dinitrotoluene                    | 90 - 395                        | 240   | 306 - 1440+  | 720+  |
| 3,5-Dinitrobenzoic acid               | 192 - 667                       | 330   | 286 - 1440+  | 720+  |
| Picric acid                           | 110 - 697                       | 430   | 278 - 1440+  | 720+  |
| 2,4-Dinitrophenylhydrazine            | 68 - 720+                       | 720+  | 468 - 1440+  | 720+  |
| P-Nitrodimethylaniline                | 163 - 720+                      | 1440+ | 720+ - 1440+ | 720+  |
| Dinitronaphthalene                    | 1440+                           | 1440+ | 1440+        | 1440+ |
| 3,4-Dichloronitrobenzene              | 1440                            | 1440+ | 1440+        | 1440+ |
| 2,4-Dinitroaniline                    | 1440                            | 1440+ | 1440+        | 1440+ |
| 2,6-Dichloronitroaniline              | 1440                            | 1440+ | 1440+        | 1440+ |

\*Each mean obtained from 25 grasshoppers treated and observed individually.

taining definite percentages of the chemicals in diatomaceous earth were prepared and adult 'hoppers were individually dipped in the different dust-mixtures. At least 50 adults of *M. bivittatus* Say (25 males and 25 females) were used in each test. The results of these experiments are given in table 2. As 3,5-dinitro-o-cresol continued to stand out as the most toxic compound, it was selected for further study.

In all of the foregoing tests, the grasshoppers were thoroughly coated with the chemical by dipping them in the dust. The excess dust was then immediately removed from their bodies by vigorously jarring the small

cylindrical screen cages as soon as the 'hoppers could be transferred to them. In the subsequent studies, a dusting atomizer was used and both 'hoppers and plants were dusted in large bell jars. Reasonably accurate dosages were obtained by weighing the amount of dust collected on measured squares of paper placed on the floors of the bell jars. In the dusting experiments, 50 grasshoppers, 10 groups of 5 each, were used in each test. As the first two series of experiments showed that the males were more easily killed, only adult females were employed in the dusting experiments.

The data presented in table 3 show that 3,5-dinitro-o-cresol, 2,4-dinitro-o-cyclohexylphenol and 25 per cent sodium arsenite<sup>3</sup> dusts act as both contact insecticides and as stomach poisons. Ten per cent 3,5-dinitro-o-cresol dust applied at the rate of 10 pounds per acre killed all of the 'hoppers in 96 hours and as the rate of application was increased the time required to kill was reduced. When applied as a contact dust (table 3, series A) 5 and 10 per cent 3,5-dinitro-o-cresol dusts gave quicker kills and more effective control than a 25 per cent sodium arsenite dust mixture. When used as stomach poisons (series B) or as both stomach poisons and contact insecticides (series C) the relative toxicity of the two compounds remained about the same.

No large-scale field tests were possible but crude fence row trials, for which no method of measuring the amount of dust used or accurately

TABLE 2. *Average time between treatment and death of Melanoplus bivittatus adults when thoroughly coated with dust mixtures containing various percentages of a chemical compound in diatomaceous earth*

| Compound                       | Concentration<br>(per cent) | Time to kill (minutes) |       |            |       |
|--------------------------------|-----------------------------|------------------------|-------|------------|-------|
|                                |                             | Male                   |       | Female     |       |
|                                |                             | Range                  | Mean* | Range      | Mean* |
| 3,5-Dinitro-o-cresol           | 100                         | 8 - 20                 | 14    | 14 - 29    | 21    |
|                                | 25                          | 14 - 25                | 19    | 16 - 58    | 32    |
|                                | 10                          | 14 - 36                | 22    | 31 - 90    | 43    |
|                                | 5                           | 14 - 46                | 23    | 33 - 89    | 45    |
|                                | 2                           | 29 - 51                | 35    | 35 - 127   | 66    |
|                                | 1                           | 45 - 130               | 65    | 65 - 396   | 171   |
| 2,4-Dinitro-o-cyclohexylphenol | 100                         | 22 - 42                | 33    | 52 - 97    | 71    |
|                                | 25                          | 71 - 144               | 83    | 156 - 424  | 252   |
|                                | 10                          | 111 - 322              | 150   | 333 - 720  | 544   |
|                                | 2                           | 100 - 675              | 508   | 420 - 1080 | 751   |
| 2,4-Dinitrophenol              | 100                         | 8 - 19                 | 14    | 17 - 34    | 22    |
|                                | 2                           | 113 - 362              | 184   | 116 - 364  | 340   |
| Sodium arsenite                | 100                         | 14 - 43                | 32    | 18 - 54    | 35    |
|                                | 25                          | 151 - 401              | 240   | 220 - 407  | 330   |

\*Each mean obtained from 25 grasshoppers treated and observed individually.

<sup>3</sup>Sodium arsenite dust kindly provided through the courtesy of Dr. Claude Wakeland of the Bureau of Entomology and Plant Quarantine.

TABLE 3. Percentages of *Melanoplus bivittatus* adult females dead 12, 24, 48 and 96 hours after treatment; when treated as shown with insecticide dusts of various concentrations

| Treatment                      |                             |                 | Hours after Treatment |     |     |     |
|--------------------------------|-----------------------------|-----------------|-----------------------|-----|-----|-----|
| Insecticide                    | Concentration (percent-age) | Pounds per acre | 12                    | 24  | 48  | 96  |
| Series A                       |                             |                 |                       |     |     |     |
| 3,5-Dinitro-o-cresol           | 10                          | 40              | 100*                  |     |     |     |
|                                | 10                          | 25              | 100**                 |     |     |     |
|                                | 10                          | 20              | 100                   |     |     |     |
|                                | 10                          | 15              | 86                    | 100 |     |     |
|                                | 10                          | 10              | 78                    | 92  | 95  | 100 |
|                                | 10                          | 7               | 72                    | 82  | 88  | 92  |
|                                | 5                           | 40              | 100                   |     |     |     |
|                                | 5                           | 25              | 76                    | 100 |     |     |
|                                | 5                           | 20              | 26                    | 60  | 82  | 84  |
|                                | 2                           | 40              | 40                    | 80  | 100 |     |
|                                | 2                           | 20              | 26                    | 56  | 60  | 62  |
|                                | 25                          | 15              | 16                    | 36  | 60  | 70  |
|                                | 25                          | 10              | 4                     | 8   | 26  | 36  |
| Series B                       |                             |                 |                       |     |     |     |
| 3,5-Dinitro-o-cresol           | 10                          | 10              | 80                    | 94  | 100 |     |
| 2,4-Dinitro-o-cyclohexylphenol | 2                           | 20              | 40                    | 60  | 94  | 100 |
| Sodium arsenite                | 25                          | 10              | 6                     | 34  | 86  | 100 |
| Series C                       |                             |                 |                       |     |     |     |
| 3,5-Dinitro-o-cresol           | 10                          | 10              | 86                    | 100 |     |     |
|                                | 10                          | 5               | 18                    | 46  | 58  | 82  |
|                                | 2                           | 10              | 24                    | 42  | 60  | 72  |
| Sodium arsenite                | 25                          | 10              | 8                     | 38  | 88  | 100 |

Series A. Grasshoppers dusted and placed on fresh, unpoisoned food.

Series B. Soybean plants dusted and undusted grasshoppers caged on the plants.

Series C. Grasshoppers and soybean plants dusted together.

\*100 per cent dead in 2 hrs.

\*\*100 per cent dead in 4 hrs.

Fifty adult females (10 lots of 5 each) used for each dust concentration.

evaluating results was available, seemed to be in general agreement with the results obtained in the laboratory.

Rough field tests with adult Mormon crickets (*Anabrus simplex* Hald.) confined on wheat stubble by metal barriers showed definitely



that crickets were considerably harder to kill than grasshoppers, but applications of 7 or 8 pounds per acre of 15 per cent 3,5-dinitro-o-cresol dust killed 30 to 40 per cent of the crickets in three hours. In two additional tests, where 20 to 25 pounds of dust were used per acre, 86 and 99 per cent, respectively, of the crickets were killed in three hours.

Young crickets, however, seem to be very easily killed by the dinitro-cresol. First instar Mormon crickets dusted under bell jars in the laboratory were consistently killed in one hour by 3,5-dinitro-o-cresol dusts when applied at the rate of 4 pounds per acre of 5 per cent dust and 3 pounds per acre of 10 per cent dust. Even lower concentrations gave rather high mortalities but the time required was lengthened. First instar grasshoppers, *M. bivittatus*, died even more quickly than the young crickets.

Various concentrations of 3,5-dinitro-o-cresol dusts were tried also on field crickets, weevils, ground beetles, tenebrionids, ants, and several species of Hemiptera and all seemed to be susceptible to the poison. In general, the concentration and quantity of dust required to kill seemed to be correlated with the size of the insect.

In the case of the chinch bug, *Blissus leucopterus* Say, over-wintered adults were consistently 100 per cent killed in 1 hour by dust mixtures applied at the rate of 2 pounds per acre of 10 per cent dust, 3 pounds per acre of 5 per cent dust, or 10 pounds per acre of 2 per cent dust. It must be kept in mind, however, that all dosages were applied to a smooth surface under laboratory conditions and that in all probability considerably higher dosages would be required in the field. The limited supply of the chemicals did not permit field test, except on a very limited basis.

#### DISCUSSION

The experimental data in Iowa suggest the possibility of obtaining good kills of grasshoppers in a comparatively short time with a 10 per cent 3,5-dinitro-o-cresol dust applied at the rate of 10 to 15 pounds per acre.

Although the practical use of dinitro-o-cresol as a dust for grasshopper control remains to be established by further study, the results reported in this paper are not inconsistent with data presented by numerous European workers, mostly in Germany, who, in the last few years, have reported varying degrees of success attending the use of dusts containing dinitro-o-cresol for the control of aphids, geometrids, nunmoth, and May beetles. Experimental work for the control of locusts and grasshoppers has been conducted also in Argentina and Africa.

Although 3,5-dinitro-o-cresol is quite insoluble, it reportedly burns considerably some types of foliage. Marcus (1937) reported spruce, larch, beech and birch moderately scorched but meadow grass uninjured. Thiem (1938) says, "Owing to injury to leaves dusting and spraying is not recommended in orchards." Schwerdtfeger (1939) observed, "Dinitro-cresol not only destroys all adults (cockchafers) with which it comes in contact, but as it also kills the young foliage the surviving adults die."



Other workers present conflicting views on the degree of burning produced possibly because of differences in the tolerance of the plants and conditions under which they worked. The sodium salt of 3,5-dinitro-o-cresol which is water soluble is used as a selective weed killer. Westgate and Raynor (1940) report success in controlling mustard, wild lettuce and other broad-leaved weeds without damage to small grains, corn, onions, alfalfa and flax. The same authors point out that the applications recommended (which are higher than would be used in insect control) have no injurious action upon the soil.

Until much more information on the plant tolerance of these chemicals becomes available, their possible usefulness may be restricted largely to the treatment of mass populations of gregarious grasshoppers and other insects where plant-burning would be of secondary consideration. At the same time, if plant tolerance will permit its use, the susceptibility of the chinch bug and many other field crop insects to this material would indicate a possible wide use for 3,5-dinitro-o-cresol on small grain or corn and even in temporary barrier construction.

The dinitro-cresol and phenol compounds are known to be powerful reducing agents and to induce increased rates of metabolism in mammals but there seems to be no agreement in the literature on the probable effect of small concentrations of these materials on man and other vertebrate animals. For the present, they should be used in field experiments only with masks and protective clothing.

Although 3,5-dinitro-o-cresol was the most toxic compound included in this study, dinitrophenol, 2,4-dinitro-6-cyclohexylphenol and the salts of these compounds also need further study because they produced a high mortality but acted more slowly than the former compound.

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# SOME REACTIONS OF GRASSHOPPERS TO CASTOR BEAN PLANTS<sup>1</sup>

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Received May 17, 1940

The wide publicity given castor bean plants, *Ricinus communis* L., as a means of grasshopper control gave rise to the laboratory experiments described below. From articles in popular magazines and newspapers it became evident that many inquiries would be received in regard to the alleged poisonous and protective powers of castor beans applicable to grasshoppers.

In 1931 the Japanese beetle was reported to have been strongly attracted to castor bean plants and killed by feeding on the leaves. Metzger (1933) stated that in cage tests the Japanese beetle fed on castor bean foliage to a limited extent only and that the plant was practically non-toxic. Tests showed that this plant was of little or no value as a trap plant for the beetle under usual field conditions. Myers (1939) summarized the insects attacking the castor bean in the United States and foreign countries. There are very few records in the literature of damage to castor bean plants by grasshoppers and only a few records of insects being poisoned by feeding on the plant.

Inasmuch as the literature has been well reviewed in a recent article by Smith (1939), an extensive review is omitted. Smith stated that grasshoppers lived fairly well on an exclusive diet of castor bean foliage and petioles but they did not relish any part of the castor bean plants for food. Since grasshoppers died in the castor bean test cages at a rate intermediate between the rate on alfalfa and that under starvation conditions, no evidence of poisoning could be observed.

## MATERIALS AND PROCEDURE

An effort was made to obtain seed of a number of varieties of the castor bean, *Ricinus communis* L. Seven varieties were obtained in the United States and an eighth secured from Europe as follows: *R. cambodgensis*, *zanzibariensis*, *scarlet queen*, *africanus*, *palma christi*, *borbonensis*, *sanguineus*, and *laciniatus* (imported). Numerous varieties were found in packages of assorted seeds, and samples of mixed seeds received contained two to five varieties each.

All nymphal stages and adults of the differential grasshopper, *Melanoplus differentialis* (Thomas), were used in the experiments. Some

<sup>1</sup> Journal Paper No. J-749 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 649.

<sup>2</sup> The writer wishes to acknowledge the assistance of Dr. C. J. Drake, under whose direction the work was conducted, also that of Dr. C. H. Richardson and Dr. G. C. Decker in checking the experiments at frequent intervals.



*Melanoplus mexicanus* (Saussure) and *Melanoplus bivittatus* (Say) also were used but the tests were less complete.

The experimental work was conducted in a greenhouse in breeding cages 1 x 1 x 2 feet with soil filled bottoms. Oats, corn, wheat, barley, and castor beans were grown in 5-inch pots. Three series of tests were made as follows, using all available varieties of castor beans in series (1) and only the eight varieties named in the foregoing in series (2) and series (3):

1. Grasshoppers were caged with a pot of cereal plant and a castor bean plant, thus allowing the 'hoppers to feed either on a crop or bean plant.

2. Adults, half-grown nymphs, and first-instar nymphs were placed in cages containing only a single variety of castor bean. For the first-instar 'hoppers, leaf discs of the castor bean were presented to the insects in glass dishes provided with moist blotting paper to prevent desiccation.

3. Newly emerged adult differential grasshoppers reared on corn and oats were transferred to screened cages (18 x 40 x 18 inches) containing eight bean plants—one of each variety. The initial population consisted of 10 males and 10 females per cage. The positions of the plants were changed at intervals within cages and varied between cages.

#### EXPERIMENTAL DATA AND RESULTS

Grasshoppers in series (1), which were given an opportunity to feed on both crop plants and castor bean plants, readily ate corn, wheat, oats, and barley in preference to any of the varieties of castor bean. Figure 1 shows the results of selective feeding when differential grasshoppers were offered oat plants and a castor bean plant in the same cage. In some cages as many as four pots of oats or other crop were consumed each day, whereas in only two cases, because of stem injury, was it necessary to replace the castor bean plant during the entire course of the experiments. The grasshoppers were not repelled by the castor beans and often rested on the leaves and stems but seldom ate any portion of the plant.

In tests of series (2), the author was unable to rear young grasshoppers on a diet of castor bean leaves. These first instar nymphs provided with castor bean food lived an average of 4.6 days as compared with 3.0 days for 'hoppers of the same age in check cages without food. Some nymphs refused entirely to eat the bean leaves, whereas others ate small amounts and a few lived to the second instar. They became quite inactive, appearing weak and unhealthy several days before death occurred.

Other *M. differentialis* nymphs were reared to the third and fourth instar stage on corn and oats before being fed exclusively on growing castor bean plants. Among these 'hoppers so limited in a diet of castor bean plants, the mortality was high. Few lived to become adults and then the adult life was short. After being restricted to castor beans, none lived beyond 30 days, whereas in the check cages, an average of 80 per cent were reared to adults, and most of them reproduced. There was no appar-

ent difference in the nutritional value of the eight varieties of castor beans as observed in these tests.

In tests of series (3), in which adult differential grasshoppers had access to the eight varieties of castor bean and no other food, some of the insects died during the first week after being placed on castor beans; half of the population was dead at the end of 29 days but a few individuals lived as long as 60 days. Throughout the tests, these adults were sluggish, quite inactive, and not easily disturbed. Death usually occurred after a day or more of feeble activity suggestive of slow-starvation diet. Feeding was light and in most cases very little of each plant was consumed. Adult differential grasshoppers showed a very slight preference for the *africanus* variety of castor bean as indicated by the number of plants destroyed. In the three cages used, the following varieties were replaced either because of stem injury or defoliation: *africanus*, 7 replacements; *palma christi*, 2 replacements; *cambodgensis* and *sanguineus*, 1 replacement of each; other varieties, no replacements.

#### SUMMARY AND CONCLUSIONS

In the tests conducted there was no evidence that grasshoppers were attracted to castor bean plants and common crop plants were selected in preference to all the tested varieties. There was no evidence also of a repellent effect of castor beans since grasshoppers used in these tests often rested on the plants and similarly under field conditions, grasshoppers have been observed to spend the night resting on castor bean plants and then move in the morning to other species of plants to feed. Because grasshoppers were able to survive longer on an exclusive diet of castor bean plants than with no food, it is concluded the poisonous principle of the castor oil plant had very little if any effect on them.

During the present grasshopper outbreak in the United States, considerable attention has been given to the castor bean plant as an alleged killing agent for grasshoppers. However, from the results of the above experiments, castor beans cannot be regarded as having any direct value in grasshopper control or as a measure of preventing grasshopper damage to crops.

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## PLATE I

Fig. 1.—(a). Cage of 100 *M. differentialis* nymphs and adults with two fresh pots of oat plants and one castor bean plant.  
(b). Same cage after six hours feeding to show comparative feeding on oats and castor bean plant. Note grasshoppers resting on castor bean plant.

## PLATE I



(a). Cage of 100 *M. differentialis* nymphs and adults with two fresh pots of oat plants and one castor bean plant.

(b). Same cage after six hours feeding to show comparative feeding on oats and castor bean plant. Note grasshoppers resting on castor bean plant.





# THE DISSIMILATION OF LEVULOSE BY HETEROFERMENTATIVE LACTIC ACID BACTERIA

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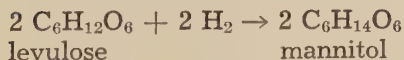
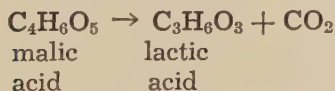
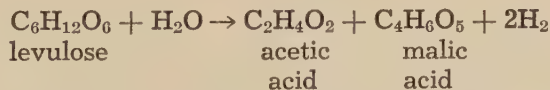
Received May 17, 1940

The heterofermentative lactic acid bacteria are unique in their ability to form mannitol from levulose. This characteristic is of interest since other hexoses, differing only slightly from levulose, are not reduced in a similar manner; otherwise, the products of the dissimilation of glucose and levulose are similar, the greatest differences being in the quantitative relationships. Considering this behavior, the question of the similarity of the kinetics of the dissimilative processes of the two sugars, is brought up.

Mazé and Perriér (1903) attempted to explain the differences between the dissimilation of glucose and of levulose through a quantitative study of the fermentation. The dissimilation of levulose was considered to be in part identical with that of glucose; that is, in the formation of lactic acid, ethyl alcohol and carbon dioxide. (Only traces of acetic acid were obtained from glucose by these authors.) Otherwise, the fermentation consisted of the reduction of levulose to mannitol with the simultaneous oxidation of ethyl alcohol to acetic acid. The alcohol was thus an intermediary in the levulose fermentation instead of a final product as in the glucose fermentation. By this method, the lower yield of ethyl alcohol and higher yield of acetic acid from levulose were explained.

Smit (1913) by a very careful quantitative study of the products of levulose fermentation, confirmed the findings of early workers as to the low yields of glycerol and ethyl alcohol.

Peterson and Fred (1920) obtained yields of mannitol accounting for as much as 70 per cent of the levulose fermented. The following schema for the fermentation of levulose was proposed:

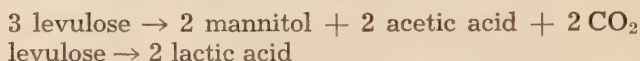


This schema requires the formation of acetic acid, lactic acid and carbon dioxide in equimolar quantities, equal to one-half the number of mole-

cules of mannitol formed. These requirements are not completely satisfied by the quantitative data of Mazé and Perriér (1903), Pederson (1929), Gayon and Dubourg (1901) and Charleton, Nelson and Werkman (1934).

Kluyver's (1935) view of the dissimilation of levulose by heterofermentative lactic acid bacteria is similar, in part, to that of Mazé and Perriér (1903). In Kluyver's scheme intermediary acetaldehyde instead of ethyl alcohol is oxidized to acetic acid.

Bolcato (1936) suggested that the quantity of lactic acid formed was independent of the quantitative relationships of the other products. The acidity produced had a marked effect on the quantity of other products. The following schema of dissimilation was formulated:



The levulose competes with intermediary acetaldehyde for hydrogen from the breakdown of intermediary pyruvic acid.

These workers seem to agree on the formation of mannitol by a reduction of levulose with activated hydrogen which results from the breakdown of another molecule of levulose. There is little uniformity in thought as to the exact reactions taking place to bring about the formation of this activated hydrogen.

A study by Nelson and Werkman (1936) in which hydrogen acceptors were added to glucose fermentations, suggests the possibility of levulose acting as a hydrogen acceptor in a manner similar to that of acetylmethylcarbinol. When acetylmethylcarbinol was added to glucose fermentations, the yields of ethyl alcohol and glycerol were decreased and the yield of acetic acid increased. The acceptor was reduced to 2, 3-butylene glycol. The differences between the glucose and levulose fermentations are similar to the differences between the normal glucose fermentation and the glucose fermentation to which acceptors had been added.

The present investigation was undertaken to study the mechanism of levulose dissimilation and to compare it with the dissimilation of glucose to which hydrogen acceptors had been added.

#### METHODS

Two heterofermentative lactic acid bacteria, *Lactobacillus lycopersici* and *Leuconostoc dextranicus* were used in the investigation.

Three series of fermentations were carried out with each organism. The first series comprised fermentations of levulose as the sole substrate, the second series employed glucose alone, and in the third series, acetylmethylcarbinol was added to the glucose fermentations.

The medium consisted of hexose, 2.0 per cent; yeast extract (Difco) 0.3 per cent; peptone 0.5 per cent;  $\text{KH}_2\text{PO}_4$  0.6 per cent and  $\text{K}_2\text{HPO}_4$  0.6 per cent; 0.5 per cent acetylmethylcarbinol was added to the medium of the third series. Solutions of (a) hexose, (b) yeast extract and peptone and (c) phosphates were sterilized 20 minutes at 20 pounds. Acetylmethyl-

carbinol was dissolved in water and sterilized by Seitz filtration. The various solutions were combined at the time of inoculation.

Inoculation was made with 50 ml. of a 3-day culture of the organisms grown on medium of the same composition to be inoculated.

The glucose and levulose fermentations were complete after 21 days' incubation at 30° C. The fermentations of glucose to which acetylmethylcarbinol had been added, were complete in about 12 days.

Complete anaerobiosis was maintained by continuously bubbling oxygen-free nitrogen through the fermenting medium.

#### METHODS OF ANALYSIS

The carbon dioxide formed during fermentation was carried into Bowen potash bulbs by a stream of oxygen-free nitrogen, bubbled continuously through the medium, and determined gravimetrically.

The volatile acids were determined by the method of Osburn, Wood and Werkman (1933) and the lactic acid by the method of Friedemann, Cotonio and Shaffer (1927).

The alcohol was oxidized to acetic acid and determined by the method of Stahly, Osburn and Werkman (1934).

Glycerol was determined by the method of Wagenaar (1911) after extracting acetone. Mannitol was extracted with alcohol and determined by the method of Smit (1914).

The hexoses were determined by the Munson and Walker (1906) method.

Acetylmethylcarbinol and 2,3-butylene glycol were determined by the methods of van Niel (1927) and Brockmann and Werkman (1933) respectively as modified by Stahly and Werkman (1936). Corrections for the effect of acetylmethylcarbinol on the determination of glucose, ethyl alcohol and 2, 3-butylene glycol were made.

Purity of the cultures was determined by microscopic and cultural examination at the time of inoculation and just before analysis.

#### EXPERIMENTAL

The products of fermentation of glucose and levulose, typical of the two organisms used, are shown in table 1, calculated as m.Mol. per 100 m.Mol. of hexose. There is a marked quantitative difference between the products of the two hexoses. A large quantity of mannitol is formed from levulose but none from glucose. The products of *Leuconostoc dextranicus* and *Lactobacillus lycopersici* are qualitatively alike but differ quantitatively. The two organisms could not be distinguished by their products of dissimilation of glucose, but *Leuconostoc dextranicus* produces less mannitol and more ethyl alcohol from levulose than *Lactobacillus lycopersici* under the conditions of these experiments.

Nelson and Werkman (1936) showed that the addition of suitable hydrogen acceptors to glucose fermentations, resulted in a decrease in the yields of ethyl alcohol and glycerol and a simultaneous increase in



TABLE 1. *Dissimilation of glucose and levulose by heterofermentative lactic acid bacteria*

|                                | <i>Leuconostoc dextranicus</i> |                    | <i>Lactobacillus lycopersici</i> |                    |
|--------------------------------|--------------------------------|--------------------|----------------------------------|--------------------|
|                                | Glucose<br>m.Mol.              | Levulose<br>m.Mol. | Glucose<br>m.Mol.                | Levulose<br>m.Mol. |
| Hexose fermented .....         | 100                            | 100                | 100                              | 100                |
| Ethyl alcohol .....            | 81.2                           | 51.2               | 74.1                             | 0.8                |
| Acetic acid .....              | 10.8                           | 34.9               | 15.3                             | 40.3               |
| Carbon dioxide .....           | 86.5                           | 77.5               | 81.0                             | 44.7               |
| Lactic acid .....              | 83.5                           | 53.4               | 83.1                             | 33.1               |
| Glycerol .....                 | 24.0                           | 2.1                | 32.6                             | 3.8                |
| Mannitol .....                 | .....                          | 29.8               | .....                            | 62.3               |
| Carbon recovery, percentage .. | 98.9                           | 99.1               | 101.1                            | 101.9              |

acetic acid. If it be assumed that part of the levulose acts as a hydrogen acceptor, and the remainder follows the same course of dissimilation as glucose, similar relationships will occur as when hydrogen acceptors are added to glucose fermentations.

To show these relationships the quantity of mannitol formed was assumed to equal the quantity of levulose acting as a hydrogen acceptor. This value was subtracted from the total levulose fermented, giving the quantity of levulose following the same course of dissimilation as glucose. The products were then calculated in terms of 100 m.Mol. of the unreduced levulose.

Typical fermentations of levulose calculated on this basis are compared in tables 2 and 3 with glucose fermentations to which acetylmethylcarbinol has been added. The reduction products of the hydrogen acceptors are now shown in these tables.

There was much less alcohol and glycerol formed from levulose by

TABLE 2. *Comparison of levulose and acetylmethylcarbinol as hydrogen acceptors in fermentations by L. lycopersici*

| Product                           | Glucose | Glucose + acetyl-<br>methylcarbinol | Levulose |
|-----------------------------------|---------|-------------------------------------|----------|
|                                   | m.Mol.  | m.Mol.                              | m.Mol.   |
| Lactic acid .....                 | 83.1    | 61.7                                | 84.8     |
| Acetic acid .....                 | 15.3    | 52.9                                | 106.7    |
| Ethyl alcohol .....               | 74.1    | 54.8                                | 2.1      |
| Carbon dioxide .....              | 81.0    | 100.1                               | 118.2    |
| Glycerol .....                    | 32.6    | 25.3                                | trace    |
| Carbon recovery, percentage ..... | 101.1   | 95.3                                | 98.3     |

TABLE 3. Comparison of levulose and acetylmethylcarbinol as hydrogen acceptors in fermentations by *L. dextranicus*

| Product                           | Glucose | Glucose + acetyl-<br>methylcarbinol | Levulose |
|-----------------------------------|---------|-------------------------------------|----------|
|                                   | m.Mol.  | m.Mol.                              | m.Mol.   |
| Lactic acid .....                 | 83.5    | 78.0                                | 76.0     |
| Acetic acid .....                 | 10.8    | 38.9                                | 50.4     |
| Carbon dioxide .....              | 86.5    | 103.1                               | 111.8    |
| Ethyl alcohol .....               | 81.2    | 67.4                                | 74.4     |
| Glycerol .....                    | 24.0    | 18.5                                | trace    |
| Carbon recovery, percentage ..... | 98.8    | 100.8                               | 98.2     |

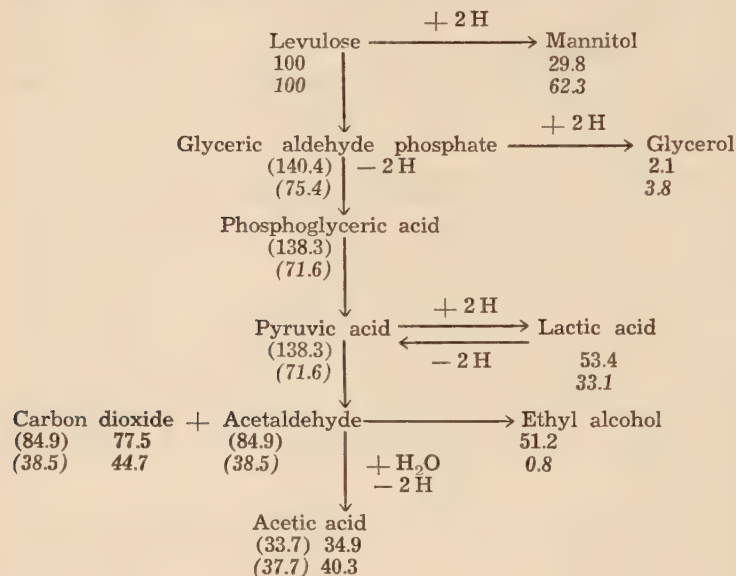
both organisms than from glucose. The quantity of acetic acid on the other hand was much greater in the levulose fermentation. These differences were more pronounced in the fermentations by *Lactobacillus lycopersici* than those by *Leuconostoc dextranicus*. The latter produced an appreciable quantity of ethyl alcohol from levulose. Whether this is characteristic of *Leuconostoc* has not been determined.

The relationships between the levulose fermentation and the glucose fermentation are very similar to those between the glucose fermentation plus acetylmethylcarbinol and the normal glucose fermentation. Assuming levulose to be an acceptor, added to a hexose fermentation, both acceptors suppressed the formation of ethyl alcohol and increased the formation of acetic acid. In the case of *L. lycopersici* the formation of ethyl alcohol was entirely prevented by levulose. The acetic acid was increased in the fermentations by *L. lycopersici* from 15 m.Mol. (glucose) to 52.9 m.Mol. with the acetylmethylcarbinol and 106.7 m.Mol. with the levulose. The ethyl alcohol decreased from 74.1 m.Mol. in the glucose medium to 54.8 m.Mol. with the acetylmethylcarbinol and 2.1 with the levulose. The same relationships exist in the fermentations by *Leuconostoc dextranicus*. Ethyl alcohol decreased from 81.2 m.Mol. (glucose) to 67.4 m.Mol. with acetylmethylcarbinol and 74.4 m.Mol. with levulose. Yields of acetic acid were 10.8 m.Mol. from glucose, 38.9 m.Mol. with acetylmethylcarbinol and 50.4 m.Mol. with levulose. These relationships indicate that levulose and acetylmethylcarbinol are playing similar roles in the dissimilation of carbohydrates. Apparently the same intermediary is oxidized in the levulose as in the "glucose plus acetylmethylcarbinol" fermentation to bring about the increase in acetic acid at the expense of ethyl alcohol. It is very likely that the same intermediate products are formed in the dissimilation of levulose as are formed in the dissimilation of glucose. These intermediates have not been definitely determined, but a schema of dissimilation is proposed in figure 1. The data from table 1 are applied to the schema. It will be noted that the values found and calculated are in good agreement.

## SUMMARY

A comparison of the dissimilation of levulose with that of glucose plus hydrogen acceptors by heterofermentative lactic acid bacteria (*Lactobacillus lypersici* and *Leuconostoc dextranicus*) indicated that the two types are similar. This relationship suggests that in the fermentation of levulose the ketose is functioning simply as an acceptor to compete with normally formed intermediary acceptors and that the mechanism of dissimilation of the levulose not acting as an acceptor is quite similar to that of glucose. In both cases, the production of ethyl alcohol and glycerol was reduced by acceptors; acetylmethylcarbinol in the case of glucose to form 2, 3-butylene glycol and levulose, acting as its own acceptor, to form mannitol. On the other hand, the yield of acetic acid was increased according to expectations.

The path of dissimilation of the oxidized levulose is apparently the same as that of glucose under normal conditions.



Values in italics are data for *Leuconostoc dextranicus*; values not italicized are for *Lactobacillus lypersici*. The values in parentheses are calculated; others are experimental.

Fig. 1. Schema of the dissimilation of levulose by *Lactobacillus lypersici* and *Leuconostoc dextranicus*.

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# NUTRITIVE REQUIREMENTS OF THE HETEROFERMENTATIVE LACTIC ACID BACTERIA<sup>1</sup>

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Received May 17, 1940

It is of deep concern that the principles of cellular nutrition be made clear, inasmuch as such knowledge is essential to an understanding of the mechanism of cell metabolism. There has been considerable renewed interest recently in the determination of the nutritive requirements of bacteria. The excellent reviews by Knight (1936), Burrows (1936) and Koser and Saunders (1938) summarize all but the latest literature in this field. Briefly the status of the problem with respect to the lactic acid bacteria may be summarized as follows: Orla-Jensen, Otte and Snog-Kjaer (1936) showed that riboflavin and one or more other "activators" are necessary for growth of certain lactic acid bacteria. They concluded from questionable evidence that one of these substances is pantothenic acid. Subsequently Snell, Strong and Peterson (1938, 1939) substantiated this conclusion by using purified preparations of pantothenic acid and showed that nicotinic acid together with pantothenic acid was essential for growth of two species in a hydrolyzed casein medium but additional factors were necessary for other species. Wood, Andersen and Werkman (1937), using a basal medium containing glucose, hydrolyzed casein, inorganic salts, vitamin B<sub>1</sub> and ether extract of yeast extract, confirmed the conclusion of Orla-Jensen et al. that riboflavin is required by the lactic acid bacteria. The activity of the ether soluble factor had previously been shown by Wood, Tatum and Peterson (1937) for the propionic acid bacteria, and by Snell, Tatum and Peterson (1937) for the lactic acid bacteria. Möller (1938) found, that in addition to the ether soluble acid and alkali labile factor of Snell et al., crystalline vitamin B<sub>6</sub> is essential. The same author (1939) established biotin as an essential factor and that in addition to vitamin B<sub>6</sub>, nicotinic acid, thiamin, riboflavin,  $\beta$ -alanine and unknown factors F (probably pantothenic acid), G and H are influential.

The amino acid requirements of the lactic acid bacteria have not been investigated extensively. Orla-Jensen et al. (1936) studied a large number of species. However, from the standpoint of determination of essential amino acids, the work is not conclusive, since the basal medium contained whey and it is doubtful whether the whey was free from traces of amino acids.

The bacteria used in this investigation were the heterofermentative lactic acid bacteria: *Lactobacillus mannitopoeus* L2), *L. buchneri* (L4) and *L. lycopersici* (L5). Under the conditions of our experiments in a

<sup>1</sup> Journal Paper No. J-690 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 421.

medium containing glucose as a source of carbon these cultures require for optimum growth; inorganic salts, amino acids, ether extract of yeast extract, thiamin and riboflavin. The ether soluble extract is not replaceable by a combination of pantothenic acid, nicotinic acid, vitamin B<sub>6</sub>,  $\beta$ -alanine, pimelic acid and uracil. Apparently the growth requirements differ from those of the species used by Snell et al. (1938) and Möller (1938), who found factors in addition to ether extract of yeast extract to be essential. According to Orla-Jensen's classification our cultures would be included in *Betabacterium*. Orla-Jensen et al. did not study the amino acid requirements of this genus. In our tests the homofermentative lactic acid bacteria (*L. casei* and *L. delbrückii*) were unable to grow in the amino acid medium, whereas, some cultures of *Streptococcus paracitrovorus* did grow. These streptococci apparently belong to the group studied by Eagles, Okulitch and Kadzielawa (1938).

The purpose of the present investigation was to determine the effectiveness of thiamin, riboflavin and ether extract of yeast extract in stimulating growth in an amino acid medium, and particularly to ascertain which amino acids are influential in promoting growth and acid production.

#### EXPERIMENTAL

*Media.* The constituents were used in the following concentrations unless otherwise stated: glucose 1.0 per cent;  $(\text{NH}_4)_2\text{SO}_4$  0.3 per cent; sodium acetate 0.6 per cent; inorganic salts,  $\text{K}_2\text{HPO}_4$  0.025 per cent,  $\text{KH}_2\text{PO}_4$  0.025 per cent,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.010 per cent,  $\text{NaCl}$  0.005 per cent,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.005 per cent and  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  0.005 per cent; hydrolyzed casein 0.15 per cent plus tryptophane 0.005 per cent or a mixture of amino acids containing 0.00375 per cent of each amino acid except cystine, which was 0.0005 per cent. The concentrations of amino acids were on the basis of the naturally occurring isomer. Thiamin was used in a concentration of .01  $\mu\text{g}$  per ml.; riboflavin .05  $\mu\text{g}$  per ml.; ether extract of 7.5 mg. of Difco yeast extract per ml.

The hydrolyzed casein was prepared by acid hydrolysis of Glaxo-casein A/E at 120°C. for 10 hours. The sulfuric acid was neutralized with  $\text{Ba}(\text{OH})_2$  and the  $\text{BaSO}_4$  removed by filtration. The final pH was adjusted with sodium hydroxide solution to 6.8. The amino acids were purchased from the University of Illinois and Eastman Kodak Company and consisted of the following: glycine, dl-alanine, dl-valine, dl-leucine, dl-isoleucine, dl-phenylalanine, l-tyrosine, l-proline, l-hydroxyproline, d-glutamic acid, l-aspartic acid, l-arginine, dl-lysine, l-histidine, dl-threonine, dl-methionine, l-cystine, l-tryptophane, dl-serine. The thiamin (Eastman) and riboflavin (Bordon) were crystalline. The ether extract of yeast extract was prepared as described by Wood, Tatum and Peterson (1937) with the exception that a large part of the succinic acid was removed by holding the extract in ether in the refrigerator for 48 hours and discarding the separated crystals. In the experiments in which replacement of the ether soluble factor by other growth factors was attempted, the medium contained the following micrograms of each substance per ml. of medium:

pantothenic acid<sup>2</sup> 0.2, nicotinic acid 2.0,  $\beta$ -alanine 2.0, vitamin B<sub>6</sub> 0.96, pimelic acid 2.0, uracil 6.0. The media were tubed in 5 ml. portions in 13 mm. x 150 mm. tubes and autoclaved at 15 lbs. for 20 minutes.

**Inoculum.** Bacteria for the inoculum were grown in the above described hydrolyzed casein medium and carried through at least three transfers before use. Centrifuged cells from a 4 or 5 day culture were washed once with a volume of water equivalent to that of the original medium and then suspended in an equal volume of water. One drop of this suspension was used per 5 ml. of medium.

**Incubation and measurement of growth.** Incubation was at 30°C. for 5 days under cotton plugs. Growth was measured by titrating the acids formed with 0.05 N alkali using bromothymol blue indicator.

**Stimulating effect of accessory factors.** Table 1 shows the influence of riboflavin, thiamin, ether extract of yeast extract and tryptophane on acid production by the three cultures considered in this investigation. Six different media were tested: (1) the complete medium alone and with the following omissions: (2) riboflavin, (3) thiamin, (4) thiamin and riboflavin, (5) ether extract of yeast extract and (6) tryptophane. The results from four serial transfers are given in ml. of 0.1 N acid per 10 ml. of medium. Consider first the results with culture L4, *Lactobacillus buchneri*. The omission of riboflavin did not retard the growth, from which it may be concluded that the compound is not an essential constituent of the medium and furthermore does not stimulate growth when present. The removal of thiamin decreased the acid production some-

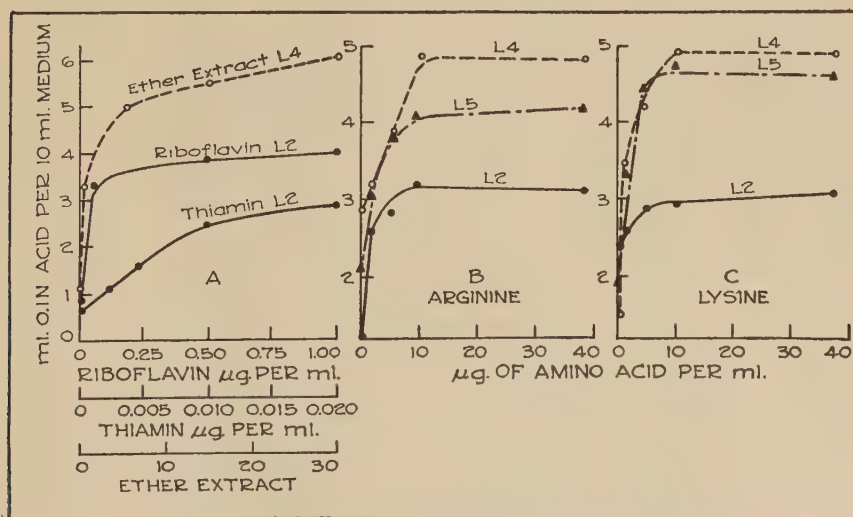


Fig. 1. The effect of variation of the concentration of accessory factors and arginine and lysine on production of acid.

<sup>2</sup> Appreciation is expressed to Dr. R. J. Williams for supplying a preparation of pantothenic acid.



TABLE 1. *Effect of riboflavin, thiamin, ether extract of yeast extract and tryptophane on acid production by the heterofermentative lactic acid bacteria in hydrolyzed casein plus tryptophane medium*

| Culture number                 | L2  |     |     |     | L4  |     |     |     | L5  |     |     |     |
|--------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                                | 1   | 2   | 3   | 4   | 1   | 2   | 3   | 4   | 1   | 2   | 3   | 4   |
| Number of transfers            |     |     |     |     |     |     |     |     |     |     |     |     |
| Compound omitted from medium   |     |     |     |     |     |     |     |     |     |     |     |     |
| None                           | 2.3 | 2.5 | 2.3 |     | 4.5 | 3.9 | 4.7 |     | 2.2 | 2.5 | 2.2 |     |
| Riboflavin                     | 2.2 | 1.9 | 1.9 | 1.9 | 4.7 | 4.8 | 5.2 | 4.0 | 1.7 | 1.8 | 1.7 | 1.9 |
| Thiamin                        | 1.8 | 1.8 | 1.8 | 1.9 | 3.0 | 3.5 | 3.9 | 3.5 | 1.3 | 1.4 | 1.4 | 1.7 |
| Thiamin and riboflavin         | 0.4 | 1.3 | 0.1 | 0.0 | 3.5 | 3.2 | 3.6 | 3.5 | 0.9 | 0.2 | 0.0 |     |
| Ether extract of yeast extract | 1.0 | 0.1 | 0.0 |     | 1.7 | 0.2 | 0.0 |     | 0.9 | 0.2 | 0.0 |     |
| Tryptophane                    | 2.6 | 2.7 | 2.8 | 3.0 | 3.4 | 1.8 | 0.9 | 0.5 | 1.8 | 2.1 | 2.7 | 2.6 |

Note: The values reported in the tables are not always comparable for different experiments, apparently because there is some variation in the activity of the bacteria. The cultures were not very active at the time of this experiment.

what but it was not essential for growth. When ether extract of yeast extract is omitted, growth is no longer obtained on serial transfer. Ether extract of yeast extract is essential for culture L4 (*L. buchneri*) and likewise for cultures L2 (*L. mannitopoeus*) and L5 (*L. lycopersici*). Both riboflavin and thiamin stimulate growth of cultures L2 and L5 but neither is essential in the presence of the other. When both compounds are omitted, continuous growth is no longer possible. Apparently thiamin and riboflavin can reciprocally replace each other in this case.

Figure 1A shows the effect of varying the concentration of the ether extract, riboflavin and thiamin. Ether extract of yeast extract approaches its full effect when the ether extract from 6.0 mg. of Difco yeast extract is added per ml. of medium. Riboflavin is almost fully effective at 0.05 microgram per ml. and thiamin at 0.01 microgram per ml. The concentration of thiamin was reduced to 0.0025 microgram per ml. in the riboflavin tests and the riboflavin to .01 microgram in the thiamin tests in order to reduce the growth at zero concentration. This procedure produces clear cut results. Also, it is sometimes helpful to use a serial transfer to deplete the carry-over in the inoculum. However, occasionally this practice is unsuccessful, inasmuch as the bacteria may adapt themselves to growth in the absence of thiamin or riboflavin during the transfers (Wood, Andersen and Werkman, 1938; Silverman and Werkman, 1939). It is essential that the inoculum consist of bacteria in a proper physiological state in order for the above influences to be shown. In our experiments this has been accomplished by carrying the cultures in the hydrolyzed casein medium for some time previous to use in the test.

*Amino acid requirements.* The determination of the amino acid requirements of bacteria is complicated somewhat by variation in the physiology of the organism. The cultures tested have shown reasonably constant requirements, probably because the inoculum always has been grown in hydrolyzed casein plus tryptophane medium, hence no demand is placed on the culture to adapt itself to growth in the amino acid deficient medium. A washed suspension of the cells has been used to minimize carry-over in the inoculum. Depletion by serial transfer was not employed in order to reduce the chance of adaptation by the organism.

The nineteen amino acids were split into the six groups shown in table 2. The first group contains the five amino acids which had proved to be essential in preliminary experiments; the second group, the basic amino acids; the third, the dicarboxylic amino acids; the fourth, the ring compounds other than tryptophane; and the fifth and sixth groups comprised the remaining less complex amino acids. A survey of the results in table 2 shows that omission of any one of the six groups caused a reduction in the quantity of acid produced. Apparently each group contains an amino acid which is necessary for optimal growth.

Inasmuch as all of the groups were essential, the effect of omission of each amino acid singly from the mixture of nineteen was determined. The results are shown in table 3. Of the total group of nineteen, only glycine, leucine, isoleucine, proline and hydroxyproline were without

TABLE 2. *Effect of omission of groups of amine acids from the mixture of nineteen*

| Amino acids omitted from the mixture of nineteen    | Culture number                     |     |     |
|---|------------------------------------|-----|-----|
|   | L2                                 | L4  | L5  |
|   | ml. of 0.1 N acid/10 ml. of medium |     |     |
| None  | 4.3                                | 6.0 | 4.5 |
| Threonine, methionine, tryptophane, cystine, serine | 0.5                                | 0.9 | 0.7 |
| Arginine, lysine, histidine                         | 1.1                                | 2.2 | 0.6 |
| Glutamic acid, aspartic acid                        | 0.7                                | 0.9 | 0.9 |
| Phenylalanine, tyrosine, proline, hydroxyproline    | 1.5                                | 4.2 | 1.4 |
| Glycine, alanine, valine                            | 0.9                                | 1.5 | 0.9 |
| Leucine, isoleucine                                 | 2.7                                | 2.7 | 2.6 |

significant effect on all three cultures. The basic amino acids did not give uniform results on all cultures except in the case of arginine. Growth was less vigorous in the absence of arginine. Omission of lysine retarded growth of cultures L4 and L5. Tryptophane apparently is not essential for cultures L2 and L5 but is for culture L4. The influence of removal of tryptophane is shown more clearly in table 1 in which the tryptophane is depleted by serial transfer. There was no growth in the case of culture L4 after the fourth transfer.

The results in table 2 show that when both leucine and isoleucine are omitted growth is poor, yet in table 3 omission of either of these amino acids singly has little effect. Apparently the two amino acids may be used interchangeably. Table 4 confirms this suggestion and shows further that glycine, proline and hydroxyproline may be omitted with no decrease in growth by the three cultures. Fourteen amino acids plus leucine or isoleucine or both give within experimental error the same results as the nineteen amino acids.

The results with the basic amino acids are less conclusive (table 3) than with the others. Therefore the possible combinations of basic amino acids were tested by addition to a mixture of the remaining twelve amino acids that had been found necessary for optimal growth. The results (table 5) show that no one of the three basic amino acids can replace the complete group for any one of the three cultures although lysine is fairly effective for culture L4. Apparently the mixture of all three basic amino acids is better than any other combination but arginine plus lysine can nearly replace the function of the three.

The effect of varying the concentration of the individual amino acids in the presence of a constant concentration of the remaining eighteen (.0037 per cent except cystine) is shown in figure 2. Glycine, isoleucine, proline, hydroxyproline and histidine were omitted in the tests with arginine and lysine (figures 1B and 1C). It is apparent that each amino

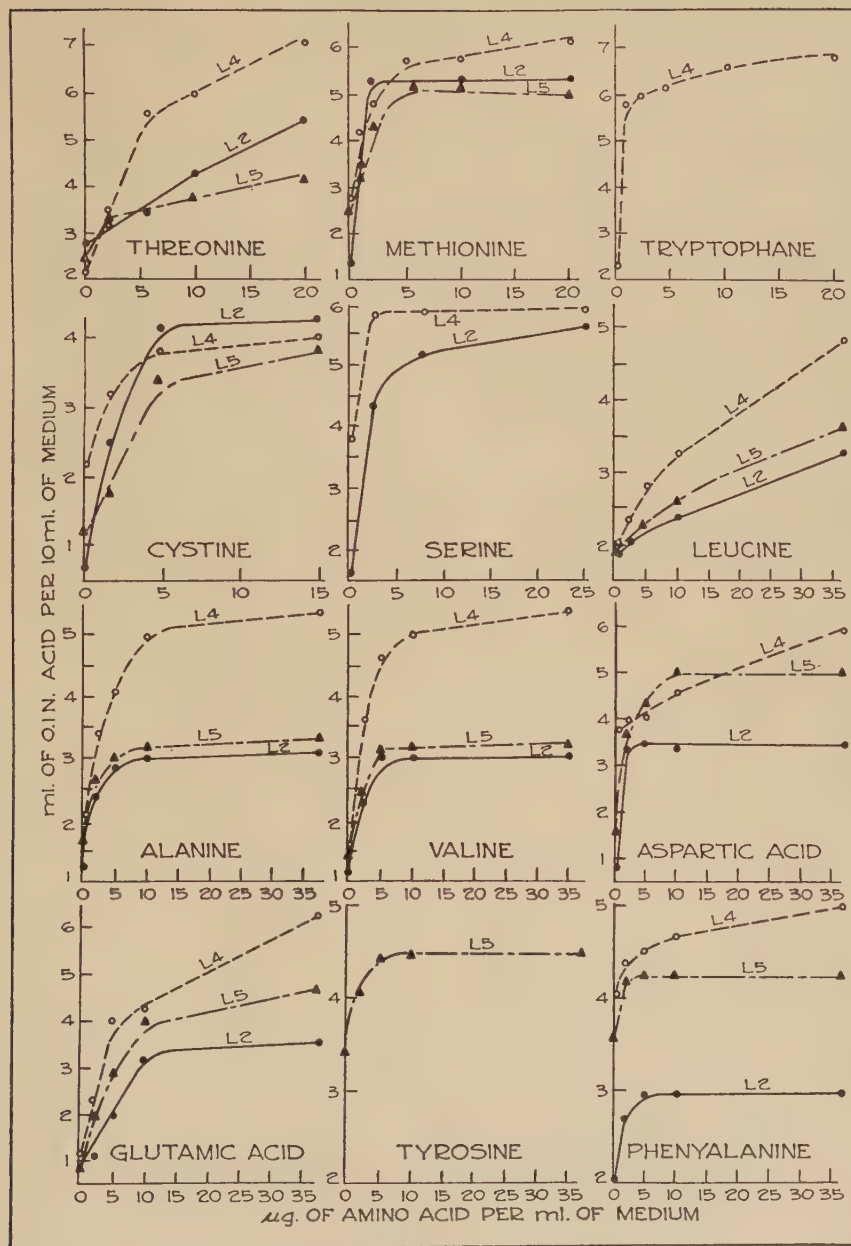


Fig. 2. The effect of variation of the concentration of amino acids on production of acid.



TABLE 3. *Effect of omission of the individual amino acids from the mixture of nineteen*

| Amino acid omitted | Culture number                        |      |     |
|--------------------|---------------------------------------|------|-----|
|                    | L2                                    | L4   | L5  |
|                    | ml. of 0.1 N acid/10 ml.<br>of medium |      |     |
| None               | 4.4                                   | 6.1  | 4.5 |
| Serine             | 1.5                                   | 4.2  | 1.7 |
| Tryptophane        | 3.9                                   | 1.6  | 3.9 |
| Methionine         | 2.4                                   | 3.8  | 2.0 |
| Threonine          | 1.1                                   | 0.8  | 1.2 |
| Cystine            | 0.7                                   | 2.1  | 1.6 |
| Arginine           | 2.8                                   | 4.0  | 3.0 |
| Lysine             | 4.1                                   | 2.4  | 3.4 |
| Histidine          | 4.1                                   | 4.4  | 4.4 |
| Glutamic acid      | 1.1                                   | 0.6  | 0.5 |
| Aspartic acid      | 0.9                                   | 2.1  | 0.6 |
| Phenylalanine      | 1.3                                   | 4.35 | 2.8 |
| Tyrosine           | 1.7                                   | 3.9  | 3.0 |
| *Proline           | 3.8                                   | 5.6  | 4.2 |
| *Hydroxyproline    | 3.9                                   | 5.4  | 4.3 |
| *Glycine           | 3.9                                   | 6.7  | 3.8 |
| Alanine            | 1.7                                   | 3.6  | 1.5 |
| Valine             | 1.2                                   | 1.7  | 1.2 |
| *Leucine           | 4.1                                   | 6.0  | 4.3 |
| *Isoleucine        | 5.0                                   | 6.4  | 4.5 |

\* Amino acid non-effective in this test for any of the three cultures.

acid reaches its maximal influence on acid production at a low concentration, approximately as follows, the quantities are expressed in micrograms per ml.: threonine 10 to 20, methionine 5, tryptophane 2, cystine 4.5, serine 2.5 to 7.5, leucine 37.5 (in absence of isoleucine), alanine 10, valine 5 to 10, aspartic and glutamic acids 10 for cultures L2 and L5 and 37.5 for L4, tyrosine 5, phenylalanine 5 to 10, arginine 10 and lysine 10. A mixture of these fourteen amino acids in the above concentrations was found to support growth almost equivalent to that obtained in the mixture of nineteen amino acids with .0037 per cent of each except cystine. There was considerable variation in the maximal yield of acid, which occurred in spite of the fact that the media were usually identical in the different experiments. These variations were caused largely by change which oc-

TABLE 4. *Effect of addition of leucine and isoleucine to a mixture of fourteen amino acids\**

| Amino acid mixture                       | Culture number                     |     |     |
|--|------------------------------------|-----|-----|
|  | L2                                 | L4  | L5  |
|  | ml. of 0.1 N acid/10 ml. of medium |     |     |
| 14 amino acids*                          | 1.9                                | 2.4 | 2.0 |
| 14 amino acids* + leucine                | 3.5                                | 6.5 | 4.0 |
| 14 amino acids* + isoleucine             | 4.1                                | 6.0 | 4.0 |
| 14 amino acids* + leucine and isoleucine | 3.1                                | 5.9 | 3.2 |
| 19 amino acids                           | 4.2                                | 6.2 | 4.1 |

\* Serine, tryptophane, methionine, threonine, cystine, alanine, valine, arginine, lysine, histidine, glutamic acid, aspartic acid, phenylalanine and tyrosine.

curred in the activity of the cultures during the two-year duration of the investigation. For this reason the ordinates are plotted to different scales in order to show the activity of the respective amino acids on a comparable basis.

#### DISCUSSION

It has been stated in the Introduction that the ether extract of yeast extract is not replaceable by a mixture of known growth factors including among others, vitamin B<sub>6</sub>, pantothenic acid and nicotinic acid. Growth is

TABLE 5. *Effect of the addition of arginine, lysine and histidine to twelve\* amino acids\**

| Amino acids added              | Culture number                     |     |     |
|--------------------------------|------------------------------------|-----|-----|
|                                | L2                                 | L4  | L5  |
|                                | ml. of 0.1 N acid/10 ml. of medium |     |     |
| Arginine, lysine and histidine | 3.5                                | 4.9 | 4.5 |
| Histidine                      | 1.5                                | 1.7 | 1.6 |
| Lysine                         | 1.8                                | 3.1 | 2.5 |
| Arginine                       | 2.3                                | 1.3 | 1.9 |
| Arginine and lysine            | 3.2                                | 4.3 | 4.1 |
| Arginine and histidine         | 2.5                                | 1.2 | 2.4 |
| Lysine and histidine           | 2.0                                | 2.2 | 2.3 |
| None                           | 1.4                                | 0.9 | 1.3 |

\* Serine, tryptophane, methionine, threonine, cystine, alanine, valine, glutamic acid, aspartic acid, phenylalanine, tyrosine and leucine.

TABLE 6. Comparison of the amino acid requirements of various organisms

| Lactic acid bacteria  | Diphtheria bacillus<br>H. Y. strain | Diphtheria bacillus<br>P. W. strain | <i>Clostridium sporogenes</i> |
|-----------------------|-------------------------------------|-------------------------------------|-------------------------------|
| Alanine               |                                     |                                     |                               |
| Valine                | Valine                              | Valine                              | Valine                        |
| Leucine or isoleucine |                                     | Leucine                             | Leucine                       |
| Glutamic acid         | Glutamic acid                       | Glutamic acid                       |                               |
| Aspartic acid         |                                     |                                     |                               |
| Cystine               | Cystine                             | Cystine                             | Cystine                       |
| Methionine            | Methionine                          | Methionine                          | Methionine                    |
| Serine                |                                     |                                     |                               |
| Threonine             |                                     |                                     |                               |
| Phenylalanine         | Phenylalanine                       |                                     | Phenylalanine                 |
| Tyrosine              |                                     |                                     | Tyrosine                      |
| Tryptophane           | Tryptophane                         |                                     | Tryptophane                   |
| Arginine              |                                     |                                     | Arginine                      |
| Lysine                |                                     |                                     | Histidine                     |
|                       | Histidine                           |                                     |                               |
|                       | Glycine                             |                                     |                               |

not completely absent, however, in the presence of these factors on the amino acid medium containing thiamin and riboflavin. In fact, cultures L2 and L4 have been carried through as many as twelve transfers in medium completely lacking in the ether extract but in no case has the growth been luxuriant. There is apparently a factor in the ether extract necessary for optimal growth of these bacteria, which is not supplied by the known factors so far tested. It is probable that pantothenic acid and some of the other factors are important for these bacteria.

Orla-Jensen et al. (1936) claim that thiamin and tryptophane are not important in the nutrition of the lactic acid bacteria. The results presented in table 1 show that this does not hold true for all lactic acid bacteria (L4). Furthermore, some lactic acid forming streptococci require tryptophane and thiamin (unpublished data). Snell, Strong and Peterson (1937) have shown tryptophane to be essential for growth of the homo-fermentative lactic acid bacteria. It is probable that Orla-Jensen et al. did not have a basal medium completely free from thiamin and tryptophane.

The amino acid requirements of the three cultures studied are more complex than found by other workers for different bacteria. Mueller (1935<sub>1</sub>), (1935<sub>2</sub>) has studied the requirements of the Ho Yu strain and the Park-Williams strain of diphtheria bacillus and Fildes and Richardson (1935) those of *Clostridium sporogenes*. A comparison of the requirements of these bacteria with those of our cultures is shown in table 6. Alanine, aspartic acid, serine and threonine were not required by either the diphtheria bacillus or *Cl. sporogenes*. The authors have found no previous record of results showing that serine or threonine is required by bacteria. Threonine is decidedly essential for optimal growth of these bacteria. This fact might well be used as the basis of a qualitative and quantitative determination of this amino acid. The extensive list of amino

acids found important for the lactic acid bacteria may be due in part to the method of determining growth. Two factors are involved in our measurement, growth and acid production. Acid production is very closely correlated with growth, as judged by the turbidity of cells. By inspection of the titrations, however, it is apparent that some of these amino acids are not essential for moderate growth. Each of the fourteen amino acids (table 6) has a definitely specific action as shown by the concentration curves (figures 1B, 1C and 2). These amino acids certainly play an important role in the physiology of these bacteria, although perhaps not in the same sense as measured by Mueller or Fildes and Richardson. Adaptation of these bacteria to a simpler amino acid medium or perhaps to a medium containing ammonium salts as the sole source of nitrogen has not been studied extensively. However, preliminary results indicate that the bacteria are rather stable in their amino acid requirements and not as readily subject to quick training to growth on simple nitrogen sources as found by Gladstone (1937) with *Staphylococcus aureus* and Wood, Andersen and Werkman (1938) for propionic acid bacteria.

#### SUMMARY

The nutritional requirements of three cultures of heterofermentative lactic acid bacteria have been studied to determine their accessory growth factor and amino acid requirements. Riboflavin or thiamin, and factors occurring in ether extract of yeast extract are necessary for maximal growth of these bacteria in an amino acid medium. Twelve amino acids: alanine, valine, glutamic acid, aspartic acid, cystine, methionine, serine, threonine, phenylalanine, tyrosine, arginine and lysine are essential; that is, omission of any one of these from the medium retards growth and acid production. In addition either leucine or isoleucine must be added, the two apparently are interchangeable. Tryptophane is essential for one culture, L4, the other two cultures can dispense with it.

Our appreciation is expressed to Mr. A. A. Andersen for assistance in some of the early experiments.

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## THE QUANTITY OF PERICARP IN SEVERAL HYBRID AND INBRED STRAINS OF SWEET CORN<sup>1</sup>

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Received June 5, 1940

The changes that occur in the outer layers of the sweet corn kernel, especially the hull, or more correctly the pericarp, are of major interest to the canner and the breeder. Toughness in canned or fresh sweet corn is generally attributed to the toughness of the pericarp. During the processing of corn for canning, the canner can control to some degree the flavor, sweetness and consistency, but no practical methods have been devised for tenderizing the kernel which at the same time do not injure quality. Of course, corn becomes tougher as it passes the prime stage of maturity, but at the prime canning stage (70-72 per cent moisture) practically all strains of sweet corn are tougher than desired.

For determining the degree of tenderness of the pericarp of sweet corn kernels, the puncture tester described by Magoon and Culpepper (1) has been used extensively. A comparison of varieties by this method is not accurate unless all kernels measured are of the same degree of maturity; that is, have the same moisture content. The sweet corn breeder can control toughness to a limited extent as shown by Johnson and Hayes (3) in a study of the inheritance of pericarp tenderness in sweet corn. They crossed a very tender open-pollinated variety and a tough pericarp Crosly inbred. The two parents used in the cross showed a consistent difference of approximately 100 units as measured with a puncture tester, while the  $F_1$  cross was intermediate in puncture test value. The same investigators found a daily increase of 20-30 units in puncture test values 18-22 days after pollination. Doxtater (2) also concluded that inbred lines showing low puncture indexes tended to produce crosses having a low puncture index.

The canner and the sweet corn breeder are not directly interested in either the very early or the very late stages of the development of the corn kernel, but an understanding is essential for interpretation of the intermediate stages.

Following fertilization the ovule and its integuments are displaced by the enlarging embryo and endosperm. Concurrent with this is the gradual change of the ovary wall into the pericarp. Ten to 20 days after fertilization, the growth of the entire kernel is very rapid; near the eighteenth day there remains only a very limited peripheral area of nucellus; the integuments as definite layers of tissue have almost disappeared, leaving here and there small masses of disintegrating protoplasm between the

<sup>1</sup>Journal Paper No. J-764. Iowa Agricultural Experiment Station. Project 607.

pericarp and the outer epidermis of the nucellus, and the pericarp has attained its maximum thickness. In the period of 20 to 40 days after fertilization, both the embryo and endosperm continue to enlarge, the epidermis of the endosperm differentiates into aleurone layer, the remainder of the nucellus disappears, except for its outer epidermal wall, and the pericarp is changed to a thin layer. In regard to integuments, it was formerly believed that in corn, like in cereals, the integument tissue persisted to maturity, forming a definite layer; but later work of several investigators (4, 5, 6) indicates that the integuments of the ovule disintegrate and virtually disappear except for scattered non-cellular, non-continuous remnants. The nucellar membrane is presumed to follow in the same manner.

The pericarp, which at the time of fertilization consists of thin-walled parenchyma cells, has increased at the end of 10 days to twice its original thickness. In the crown region of the kernel, the pericarp increases in thickness up to the ninth or twelfth day, and in the basal region to the fifteenth or eighteenth day. About 10 days after fertilization, the middle cells of the pericarp begin to disintegrate in the crown, thereby forming an inner and an outer region or epidermis. Gradually this partial collapse in the middle region extends down the sides of the kernel. After the twentieth day, the outer pericarp increases in size by developing relatively thick cell walls, the cells remaining intact as the kernel expands. In contrast, the cells of the inner pericarp, failing to increase in size, become separated. For a while the cells of the inner epidermis elongate sufficiently to maintain their continuity as a layer of cells, but eventually even these are drawn apart laterally. In the final stage the tangential walls of the outer pericarp continue to thicken, and compression of the entire pericarp occurs, first of the inner layer and then of the entire tissue, to form a tough protective covering. The following problem was undertaken to determine if there were differences in the amount of pericarp between tender and tough strains of sweet corn at the edible or canning stage.

#### PROCEDURE

The ear corn was husked and all the silk was picked from the ear. A razor blade or other sharp cutting instrument was used to cut the corn one row at a time just above the tipcap. Slightly more than 1000 grams of kernels were cut off the cob and thoroughly mixed to insure uniform sample. Two moisture samples, consisting of approximately 60 grams each, were weighed out and dried to constant weight in a vacuum oven at 75° C. and approximately 80 mm. pressure.

An 800-gram sample was taken for the pericarp analysis. The sample was first washed with distilled water by rubbing the kernels together lightly in a pan of water. A single layer of kernels was spread on oilcloth and lightly crushed to free the germ from the kernel. The loose germs were then quite readily removed by washing with a rotary motion under a stream of water in an evaporating dish covered with a 4-mesh screen having a metal side 1 to 2 inches deep. About 200 grams of the sample



were used in each washing. Since the value of this procedure depends to a large extent upon securing comparable results, it was necessary to standardize the procedure. However, the standardization was flexible enough to allow for the difference in tenderness and toughness of the samples. The most important part of the procedure was the separation of the endosperm and aleurone from the pericarp. As the endosperm and aleurone were separated by mechanical force, it was necessary to use great care in keeping the pericarp from being ground up at the same time.

The separation of pericarp from endosperm and aleurone took place in a food press which was operated by hand. Instead of grinding all the samples the same length of time, they were ground in the food press until nearly free of endosperm and aleurone. With tough pericarp samples, it was necessary to grind for a longer time as compared to medium or tender pericarp samples. In this separation, factors to be considered are as follows: (a) The amount put in the food press at one time. (b) The length of time of grinding between intermittent washings. (c) The amount of liquid in the food press. (d) The even distribution of sample on the walls so that there is no excess pressure on any one part of the samples. (e) The effect of pressure on the wooden piece of the apparatus. In this treatment it was desired to remove as much endosperm and aleurone as possible without loss of any pericarp. It was impossible, however, to remove satisfactorily all of the endosperm and aleurone from the pericarp in the food press. Next the pericarp was placed in a 500 cc. salt-mouth bottle containing approximately 20 medium sized ball mill pebbles and nearly full of water.

The ball mill consisted of a tumbling machine so constructed that the reagent bottles traveled end-wise through a radius of about 8 inches, at the rate of 41 R.P.M. In this operation the pebbles, falling from one end of the jar to the other during the grinding period served to loosen bits of remaining endosperm and aleurone layer from the pericarp. After grinding, the samples were washed with 4 or 5 portions of distilled water through a conical 8-mesh sieve. In this way all of the remaining particles other than pericarp were removed from the residue which was then washed free from the pebbles on a 14-mesh sieve, pressed into a flat metal pan and dried to constant weight along with the moisture samples. Official methods were used for moisture in the air-dry sample, ether extract, crude fiber and total nitrogen.

The hybrids and inbreds were classified into four classes as follows: tender, medium tender, tough and very tough. This was an arbitrary classification based, according to the judgment of the writers, on chewing tests or on the general reputation of the material in the canning and the seed trade. Corn is known to be tougher to chew as it matures. The same variety appears to be tougher at 66-68 per cent than at 70-72 per cent moisture. The increase in toughness is generally attributed to increased toughness of the pericarp or increase in the quantity of pericarp.

In table 1 the data show that an increase in maturity in the case of Minhybrid 202 was accompanied by an increase in the percentage of peri-



carp. On the other hand, a strain classified as tough might contain a high percentage of pericarp as shown by inbred 13, or a strain might be classed as medium tender and have a lower percentage of pericarp at 68 per cent moisture (old) as shown by inbred 1445.

TABLE 1. *The percentage of pericarp present in several sweet corn inbreds and hybrids in 1938 and 1939*

| <i>Tender</i>           | 1938       |                         | 1939       |                         |
|-------------------------|------------|-------------------------|------------|-------------------------|
|                         | Percentage |                         | Percentage |                         |
|                         | Moisture   | Pericarp oven-dry basis | Moisture   | Pericarp oven-dry basis |
| 5. Ioana .....          | 71.1       | 5.6                     | 70.8       | 4.3                     |
| 13. Iogent 27 .....     | 73.1       | 6.8                     | 67.8       | 5.2                     |
| 15. Golden Cross Bantam | 70.7       | 4.4                     | 74.6       | 4.8                     |
| 17. Inbred 1627 .....   | 72.4       | 4.5                     | 68.4       | 4.5                     |
| <i>Medium tender</i>    |            |                         |            |                         |
| 1. Minhybrid 201 .....  | 74.1       | 5.6                     | 78.0       | 5.1                     |
| 12. Iogent 12 .....     | 70.1       | 6.4                     | 68.5       | 5.8                     |
| 18. Inbred 1445 .....   | 68.0       | 5.8                     | 68.2       | 5.3                     |
| 8. Inbred 191 .....     | 69.8       | 5.6                     | ...        | ..                      |
| <i>Tough</i>            |            |                         |            |                         |
| 2. Minhybrid 202 .....  | 74.6       | 5.3                     | ...        | ..                      |
| 3. Minhybrid 202 .....  | 72.2       | 6.1                     | 72.9       | 6.3                     |
| 4. Minhybrid 202 .....  | 66.4       | 7.1                     | ...        | ..                      |
| 6. Iogold 13 x 45 ..... | 73.7       | 6.1                     | 75.7       | 6.4                     |
| 11. Inbred 13 .....     | 75.0       | 6.4                     | 77.1       | 6.0                     |
| 14. Inbred 1214 .....   | 72.5       | 5.5                     | ...        | ..                      |
| <i>Very tough</i>       |            |                         |            |                         |
| 10. Inbred T. P. ....   | 74.6       | 8.4                     | 78.1       | 8.5                     |
| Inbred 1620 .....       | 66.6       | 7.5                     | 71.5       | 7.7                     |

This method used for determining the amount of pericarp gives comparisons between strains irrespective of moisture content within the range when corn is commonly used for canning. The kernels of tender strains contain 4-5 per cent pericarp, medium tender from 5-6 per cent, tough from 6-7 per cent and very tough over 7 per cent on a dry weight basis. The 1939 results fit into this classification with one exception. The 1938 results were not quite so consistent, but the technique of sampling and separation of the pericarp had been improved in 1939. Tough and tender pericarp strains of sweet corn differ in the quantity of pericarp present.

## SUMMARY

1. A method is presented for separating the pericarp of sweet corn from the other tissues of the kernel at the edible stage.
2. Inbred and hybrid strains of sweet corn differ in the quantity of pericarp at the canning stage.
3. Strains classed as very tough on chewing contain from 50 to 100 per cent more pericarp than those classed as tender.

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# THE RESPONSE OF THE PLUM GROWN UNDER HILLCULTURE CONDITIONS TO MODIFICATIONS IN CULTURAL TREATMENT<sup>1</sup>

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Received June 7, 1940

Systematic hillculture under the Dutch name—*bergculture*—has been practiced in the Dutch East Indies for many years. When farming first began there, it was found that the heavy rains and the steep erosive soil made it necessary to use some other system than clean cultivation. It was considered so important to develop a better agricultural system that private land owners began experimenting with combining types of plants to keep the soil almost completely covered at all times. Terracing and furrowing methods were used to insure the conservation of soil and water.

In the United States the purpose of the experiments is to develop for steep eroded soils a rational system of erosion-control farming based on the use of superior plants, so managed as to improve the soil, conserve water, and produce permanent annual yields and reasonable profits.

In the extensive erosion area of southern Iowa and northern Missouri one of the immediate problems seemed to be the development of a cultural system based on the foregoing principles, for the utilization of a portion of the steep eroded land of the area to raise an adequate supply of fruit for local use. Of the tree fruits considered, the plum was one selected for experimentation because its flexibility in habitat requirements gave promise of adaptation to a wide range of modification in cultural treatment.

## MATERIALS AND METHODS

The plantings are located on the Hillculture Experimental Farm in Davis County in southeastern Iowa. The plums were planted on approximately five acres of land in three fields located on southeast, northeast, and east slopes of 10 to 30 per cent. The soil type at the top of each slope is Clinton and the remainder is Lindley. The farm had been abandoned because of excessive erosion, the depth of the remaining A horizon on the selected slopes varying from zero to ten inches.

The trees were planted at intervals of 13.2 feet on furrows on exact contours 10 feet to 20 feet apart. For each row two furrows were thrown down the slope with a tractor-drawn 16-inch plow. The lower furrow had a depth of approximately eight inches. The upper furrow, on top of which the trees were planted, was approximately four inches deep.

Before the furrows were made in the early spring of 1938 the plant cover of the slopes varied from a thin cover of bracted plantain and rag-

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<sup>1</sup> Journal Paper No. J-758 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project 582.



weed, through a mixture of bracted plantain, aster and goldenrod, to a medium dense stand of Canadian and Kentucky blue grass, sweet clover, and clover. At no time since the furrows were made has more than 10 per cent of the rainfall been lost from the slopes and for most of the time the entire precipitation of about 32 inches per year has been retained. During the two seasons of the experiment the original vegetation remaining between the rows of trees has materially increased in density. Plate I shows the general appearance and aspect of the northeast slope.

The plum varieties used were the Green Gage variety of *Prunus domestica* and the following Minnesota Agricultural Experiment Station improved varieties which have been produced from crosses between American and Asiatic plums: Monitor, Superior, Tonka, and Underwood. The trees were commercial stock raised at Shenandoah, Iowa, and were 11/16 inch, 5-7 feet, well branched trees, pruned to five 6-inch branches.

The trees were planted (April 18-22, 1938) in plots of three contour rows, each row containing 5 or 6 trees of a given variety. The three-row units vary on the slope from a length of two plots to a length of six plots. The location of the five varieties was randomized except that there were fewer plots of Green Gage and Tonka than of the other three varieties. Since the Minnesota varieties of plums are poor pollenizers, an adequate number of single pollenizer plums were put in at the end of the middle contour rows of the plots.

In contrast to the arrangement of the trees in plots of three by five or six trees, the treatments were modified row by row; each contour received the same treatment for its entire length. Each plot of 15 or 18 plants of the same variety had three different treatments and each complete treatment row covered from three to six varieties.

All of the treatments received at least minimum culture, which consisted of planting on the top of the upper furrow and hand hoeing during the growing season to a 1.5-foot radius. The treatments were as follows: (1) The original plant cover adjacent to the row was undisturbed for at least four feet with 3-foot plow strips on each side of the row for interplanting of strawberries, cane fruits, sweet potatoes, asparagus, etc.; (2) The original plant cover on each side of the original double furrow was disked twice each season; (3) The original plant cover on each side of the original double furrow was left undisturbed; (4) The soil in the bottom of an extra furrow, about one foot above the row, was loosened to a depth of 2.5 feet, comparable to the action of a 6-inch chisel plow; (5) Two extra furrows were plowed above the row of trees and kept cultivated; (6) The row of trees was plowed and cultivated once above and below for a distance of at least four feet and kept mulched with the exception of a small open furrow on each side; and (7) The row of trees was plowed and kept cultivated above and below for a distance of at least four feet on each side.

#### RESPONSE OF THE TREES TO VARIATIONS IN TREATMENT

In spite of the fact that the varieties had been tested for conditions comparable to those of southern Iowa, there was a definite variety dif-

ference in survival and growth (table 1). At the end of the first growing season survival was satisfactory for all varieties except Tonka, which showed a survival of only 60 per cent.

The trees planted in unfurrowed rows for comparison with those planted on contour furrows had only minimum culture and were compared with the minimum culture treatments on the contour furrows. The trees on the contour furrows showed by their response that they had an advantage in growth. A properly designed experiment for the purpose of obtaining quantitative data on this phase of the problem was set up in May, 1939.

Differentiated response of the young plum trees to variations in cultural treatment during the first growing season was negligible. This was expected since the trees, which were three years old and sturdy, probably contained sufficient reserve materials to make adequate growth under a wide range of growing conditions. There seemed to be little difference in height and branch growth between those trees which were expanding chiefly at the expense of stored food reserves and those which were becoming well established and were producing adequate supplies of reserve materials.

TABLE 1. *Survival and growth response of the five plum varieties tested, 1938*

| Variety    | Survival<br>(percentage) | Length permanent<br>branches per plant<br>(inches) | Average height<br>increase<br>(inches) | Rank |
|------------|--------------------------|--|--|------|
| Green Gage | 93                       | 32   | 5.3                                    | 4    |
| Monitor    | 95                       | 72   | 7.3                                    | 3    |
| Superior   | 100                      | 128  | 11.4                                   | 1    |
| Tonka      | 60                       | 29   | 4.3                                    | 5    |
| Underwood  | 98                       | 86   | 7.9                                    | 2    |

Figure 1 shows the difference in average number per plant of permanent branches during the two years. In number of permanent branches formed the first year, there was no measurable difference which could be attributed to variations in treatments. However, an increase in total height growth per tree of the chisel-furrow and the complete cultivation treatments was evident during the first year of growth (figure 3) probably because response in height growth seems to follow improved conditions more closely than does response in number of branches or in total length of branches.

During the second growing season (figure 1) the average number of branches per plant increased for each treatment over that of the first season. The magnitude of the increase seems to be progressively greater with cultural methods from minimum to complete cultivation. It was noticed also that at the end of the second season the plants of a given treat-

ment were more nearly alike in size and habit of growth than at the end of the first season. This is probably attributable to the fact that the individual differences in food reserves of the trees when planted tended to be equalized during the two seasons by growth of the plants under similar cultural conditions.

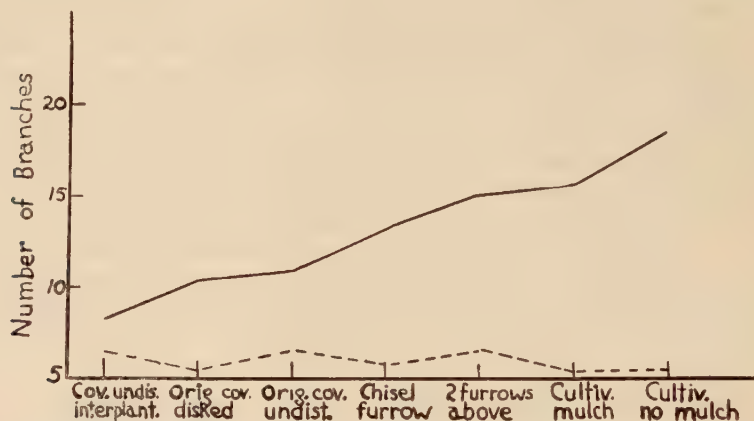


Fig. 1. Average number per plant of branches over 6 inches in length of all varieties on all slopes, 1938 ....., and 1939 .....

Three of the measurements of plant response used are compared in figure 2. The correspondence in the three measurements used to indicate response of the plants to different treatments is close enough that they might, in most cases, be used interchangeably. A graph made of the mean of the three curves (figure 2) may be used to good advantage as a curve of average plant response.

An average plant response curve of this type made from figure 2 shows that there is an increase in growth from left to right. However, there is little difference among the three minimum culture treatments on the extreme left. The competition with the original cover next to the trees seemed to be sufficient to suppress the growth of the trees, whether or not the cover was disked. Disking was not necessary to hold water on the slope since it was held by the furrows.

There is a definite increase in rate of growth from the three minimum culture treatments on the left (figure 2) to the three medium culture treatments, immediately to the right. These three treatments, the chisel furrow, two furrows kept cultivated above, and cultivation with mulch, seem to result in adequate growth response and also offer promise of adequate soil protection and soil building. Average response was higher for complete cultivation than for any of the other treatments, but soil losses into the bordering furrows and beyond make this method impracticable.

In figure 3 is shown the variation in response of the plums induced by the complex of factors represented by three different sites. While the average degree of slope varied little for the three sites, direction of slope and



general climatic factors were different. However, the greatest difference among the three sites was the degree of erosion and the density and vigor of growth of the original plant cover.

The A horizon of the soil on the northeast slope varied in depth from six to ten inches, which during the second year supported a dense growth

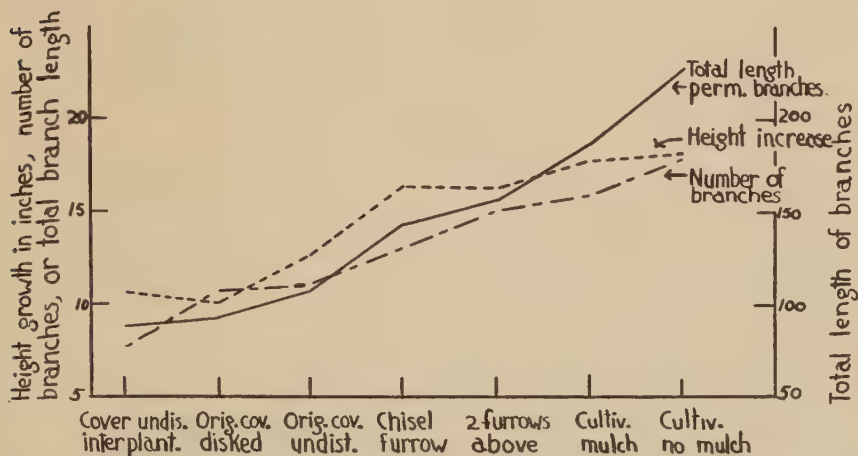


Fig. 2. Comparison of the response of the plum trees in terms of total length of permanent branches, increase in height, and total number of branches. Average of all trees on all sites. 1939.

of bluegrass, sweet clover, and red clover. In contrast, the A horizon of the southeast site was almost entirely removed except near the bottom. The vegetation was sparse and represented an early stage of the succession: the ragweed-bracted plantain stage. The soil of the east slope was about half way between the other two in degree of erosion, and with original cover of the late weed-early bluegrass stage.

The trees on the northeast slope showed poor height growth with minimum culture (figure 3) and improved very little with chisel-furrow treatment, but gave good results where the competition of the original cover was reduced by plowing and the trees were either mulched or kept cultivated. Approximately the same results were obtained with minimum culture on the east slope, but the fertility of the soil was sufficiently low that release from competition by mulching and clean cultivation resulted in less tree growth than on the fertile soil of the northeast slope. On the southeast slope the trees did better under minimum culture where the water was held by furrows and the thin layer of top soil was not disturbed by cultivation. The poor response to plowing and mulching and to cultivation can probably be attributed to the low fertility of the soil and to the fact that the turning over and loosening of the small quantity of top soil resulted in excessive leaching if not to loss of fertility by erosion. There was also a greater increase in the response in total length of perma-



nent branches of cultivated trees on the east and northeast slopes than on the southeast slope.



Fig. 3. Average height growth per plant of all varieties on each of the three sites for the growing season of 1939 compared with the average for all varieties and all sites, 1938.

#### SUMMARY

1. Five selected varieties of commercial plums were planted in 1938 on contour furrows on steep eroded soil of the Lindley and Clinton types in southern Iowa.
2. The variation in the response of the trees to seven different cultural treatments on the contour furrows and to minimum culture without furrowing was greater the second year than the first.
3. Three different measurements of plant response seem to give about the same differences between treatments: (1) total length of permanent branches; (2) height increase; (3) number of branches.
4. Medium cultural treatments seemed to be superior to minimum culture in growth and establishment of the trees.
5. Complete cultivation resulted in some increase in growth but seems impracticable on the steep erosive soils used in the experiment.
6. Fertility, degree of erosion, aspect and degree of slope, and competition of the original plant cover are all important in the selection of the best cultural treatment to use.

#### PLATE I

Commercial plums planted on the contour of a northeast slope in April, 1938. July, 1939.

PLATE I





# MOTILITY OF THE EXCISED FORE-GUT OF PERIPLANETA AMERICANA (ORTHOPTERA) IN VARIOUS SALT SOLUTIONS

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Physiological salt solutions have been perfected for many different animals, but there is little available information about balanced salt solutions for insects. A fluid which will maintain insect tissues in a satisfactory condition for several hours should prove useful in the furtherance of both insect physiological and toxicological knowledge. This paper reports the results of muscular activity in various salt solutions registered kymographically by 450 excised crops from the cockroach, *Periplaneta americana*. The series of experiments described here is the first step of an attempt to produce an insect Ringer's solution for the American roach. The investigations will be continued in order to determine the specific effect of the separate ions.

Several insect physiological solutions have been tried. In 1917, Brocher (2) investigated heart contractions of *Dytiscus marginalis* in a balanced salt solution, but neglected to report its composition. In the same year, Glaser (5) grew hemolymph cells from larvae of *Malascoma americanum*, *Cirphis unipuncta*, *Laphygma frugiperda*, and *Porthetria dispar* in a Locke solution which he stated was an isotonic salt mixture. In a study of the movement of malphigian tubules from *Drosophila funebris* and *Calliphora erythrocephala*, Eastham (4) employed NaCl solutions of several concentrations. Hobson (6), in 1928, reported an extensive study of excised crop and esophageal contractions from *Dytiscus marginalis* during which he used a simple modified Ringer mixture.

In 1928, Levy (7) attempted to find an adequate solution in which to study heart preparations of *Phormia regina* and *Calliphora erythrocephala*. He made a stock solution (108 grams of NaCl, 8.5 grams of KCl, 5.5 grams of CaCl<sub>2</sub>, 2.0 grams of NaHCO<sub>3</sub>, and 0.1 gram of NaH<sub>2</sub>PO<sub>4</sub> in 10 liters of solution), tried various dilutions, and reported a twelfth dilution as giving the best results. Yeager and co-workers (14, 15, 16) have used a slightly modified Levy's solution when experimenting with contractions of heart and malphigian tubules from *Periplaneta americana*, *Prodenia eridania*, and *Blatta orientalis*.

Slifer (9), in 1934, used a solution, which had previously been reported by Belar (1), as a medium in which embryos of the grasshopper *Melanoplus differentialis*, were grown. Belar had originally devised this solution for studies of mitoses in excised testes from *Chorhippus lineatus*. While experimenting with the effects of certain drugs on crop contractions of *Dytiscus marginalis*, TenCate (11), in 1929, used a 0.95 per cent NaCl solution.



Table 1 gives a summary of the concentrations of the constituents in the solutions listed in the foregoing.

#### METHODS AND MATERIALS

The apparatus for maintaining and recording crop activity in experimental salt solutions is diagramed in figure 1, and described below. The solution to be tested was placed in an aspirator bottle (B) where oxygen was bubbled through it before and during each trial. The fluid ran through inlet tube (I) into the crop chamber which was made of a large glass tube (T). Cork (C) sealed with paraffin closed the chamber at one end. Clamp (Cl) closed off the outlet tube (O) so that the solution was drained out through overflow tube (OO). Rate of flow was regulated by stop cock (S). The excised crop (Cr) was suspended by two silk threads (ST) between a wire hook (H) and wax (W) on the end of the capillary glass recording lever (RL). Contractions were recorded on a kymograph (K) which stood upon a leveling table. (Figure 1.)

To remove the crop, the roach was first inactivated with ether vapors. After the legs had been removed close to the body wall, the specimen was fastened, ventral side up, in a small wax-bottom dissecting pan where it was immersed in some of the particular solution to be tested. The ventral body wall was carefully removed so that the entire digestive tract, from esophagus to hind-gut, was exposed. After cutting the remaining muscle and tracheal connections to the head and the crop, the fore-gut was severed from the hind-gut just posterior to the gizzard and was removed from the body. By means of a slip knot, one short thread was tied to the gut in the region of the ventriculus. No perceptible difference in crop activity was evident when the attachment was either immediately anterior or immediately posterior to the gizzard. Another longer thread was attached to the antennae. With a loop at the loose end, the shorter thread was fastened to a hook in the crop chamber. The longer thread was attached to the recording lever adjusted in length so that the actual amplitude of contraction was magnified seven times. A flow of physiological solution was maintained through the chamber at a rate of approximately 500 cc. per hour. Oxygen was bubbled through the solution in the stock bottle for the duration of each trial. All this work was done at room temperature, between 20°-26°C.

Each preparation was maintained until failure of the crop to contract terminated the experiment. Since the kymograph drum made one revolution in 16 hours, the duration of activity could be calculated in hours. Measures of amplitude were made in centimeters at intervals of one centimeter (or about every 20 minutes) throughout the time of activity. From these figures the mean amplitude was determined. Multiplication of the average amplitude and the duration of contractions yielded a product which was used as a measure of the activity of the individual crop and, consequently, as a criterion of the adequacy of a given solution. Since it was necessary to use a very slowly revolving kymograph drum, it was impossible to measure frequency of contraction in the present work.

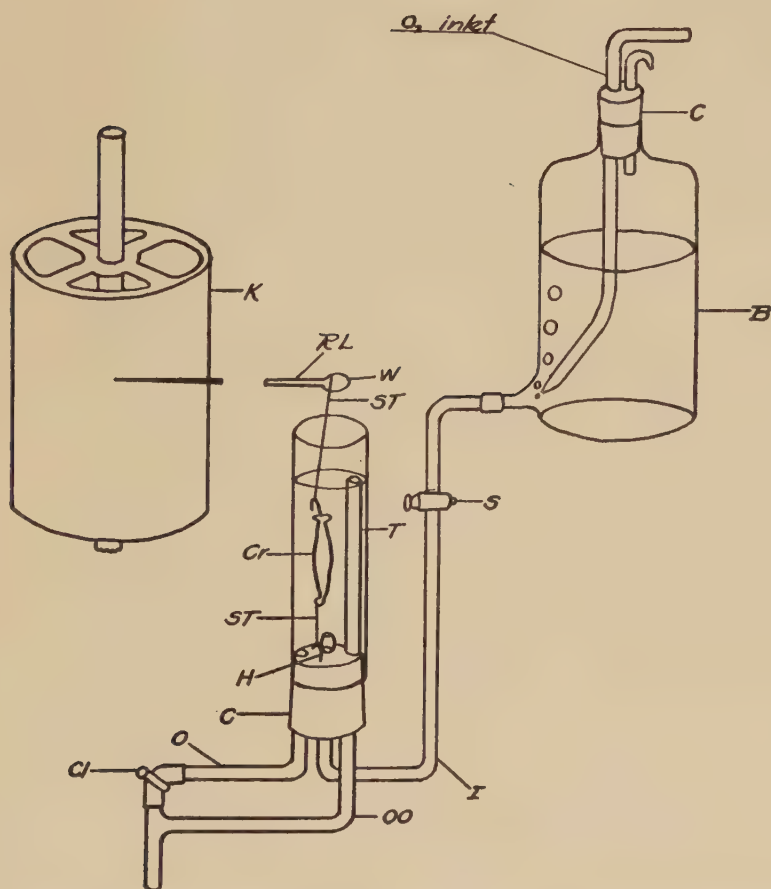


Fig. 1. Diagram of an apparatus for recording movement of the excised fore-gut of the American cockroach. (See text for complete description.)

TABLE 1. *Insect physiological solutions*

| Author                    | Date | NaCl<br>g/l | KCl<br>g/l     | CaCl <sub>2</sub><br>g/l | NaHCO <sub>3</sub><br>g/l | NaH <sub>2</sub> PO <sub>4</sub><br>g/l | MgCl <sub>2</sub><br>g/l | Glu-<br>cose | Pep-<br>tone |
|---------------------------|------|-------------|----------------|--------------------------|---------------------------|---|--------------------------|--------------|--------------|
| Brocher                   | 1917 |             | (not reported) |                          |                           |   |                          |              |              |
| Glaser                    | 1917 | 9.00        | 0.42           | 0.25                     | 0.20                      |   |                          | 2.50         | 2.00         |
| Eastham                   | 1925 | 7.50        |                |                          |                           |   |                          |              |              |
| Eastham                   | 1925 | 6.00        |                |                          |                           |   |                          |              |              |
| Hobson                    | 1928 | 9.22        | 0.22           | 0.22                     |                           |   |                          |              |              |
| Levy                      | 1928 | 9.00        | 0.71           | 0.46                     | 0.17                      | 0.01                                    |                          |              |              |
| TenCate                   | 1929 | 9.50        |                |                          |                           |   |                          |              |              |
| Slifer (Belar)            | 1934 | 9.00        | 0.20           | 0.20                     | 0.20                      |   |                          |              |              |
| Yeager and Hager          | 1935 | 9.82        | 0.77           | 0.50                     | 0.18                      | 0.01                                    |                          | 1.00         |              |
| Yeager, Hager and Straley | 1935 | 9.00        | 0.70           | 0.46                     |                           | few drops                               |                          | 1.00         |              |
| Yeager                    | 1939 | 10.93       | 1.57           | 0.85                     |                           |   | 0.17                     |              |              |
| Yeager                    | 1939 | 11.78       | 0.92           | 0.66                     |                           |   |                          |              |              |

(See Plate I.) Therefore, only amplitude and length of time of activity were used to calculate the activity product, expressed in centimeter-hours.

Ten crops, five from males and five from females, were tested in each solution. Preliminary determinations were made with the solutions as recommended by Slifer (9), TenCate (11), Hobson (6), Yeager (14), and Glaser (5). Using the best results from these solutions as a basis for composition of the various experimental solutions, NaCl and NaHCO<sub>3</sub> concentrations were established at 9.75 and 0.19 gram per liter of solution, respectively. The KCl and CaCl<sub>2</sub> concentrations were varied from 0.0 to 1.19 grams per liter for the former salt and 0.0 to 0.89 gram per liter for CaCl<sub>2</sub>. Three of these latter mixtures, with the addition of 0.19 gram of NaHCO<sub>3</sub> per liter, were used for additional experiments. For each of these three KCl-CaCl<sub>2</sub> ratios (0.67-0.33, 0.45-0.50, and 0.22-0.22 gram per liter) six NaCl levels (4.87, 9.75, 11.31, 12.87, 14.63, and 16.19 grams per liter) were tested in an attempt to determine the optimum NaCl concentration.

The experimental animals were adult specimens of the American roach, *Periplaneta americana*, reared in the laboratory at room temperature, and fed a diet of whole wheat bread and banana, supplemented occasionally with hamburger.

#### RESULTS AND DISCUSSION

Yeager (12) observed three types of proventricular movement in intact *Periplaneta fuliginosa*: peristaltic, antiperistaltic, and contractions involving the posterior third of the crop. These same types of movement were readily observed in the excised fore-gut of the American roach. Often peristaltic or antiperistaltic waves passed down or up the crop without visible effect upon the recording lever. The pulling contractions of the posterior third of the crop accounted for the majority of the recorded movements.

Results from each lot of ten crops in the 43 solutions are tabulated in table 2. The following information is listed for each solution: (1) concentrations of NaCl, KCl, and CaCl<sub>2</sub> in grams per liter; (2) the KCl/CaCl<sub>2</sub> ratio; (3) the average amplitude, in centimeters, of contraction for the ten crops in each solution; (4) the average length of time, in hours, of activity for the ten crops tested in each mixture; (5) the average activity products in centimeter-hours for crops from males and females; and (6) the average activity product of all crops in each solution. All solutions contained approximately 0.19 grams of NaHCO<sub>3</sub> per liter of solution, with the exception of Hobson's and TenCate's which contain none. (Table 2.)

Examination of the survey data from solutions containing from 9.00 to 10.00 grams of NaCl per liter showed that only five (22, 23, 20, Belar's, and Hobson's) produced activity products which indicated that they were at all satisfactory, both in regard to average amplitude and length of time to activity (see table 2). Of these, solution number 22 produced



TABLE 2. *Rating of solutions according to average activity products from ten crops\**

| Solution | NaCl  | KCl  | CaCl <sub>2</sub> | KCl               | Ampl.<br>(cm.) | Time<br>(hr.) | Activity Products |       |       |
|----------|-------|------|-------------------|-------------------|----------------|---------------|-------------------|-------|-------|
|          |       |      |                   | CaCl <sub>2</sub> |                |               | ♂                 | ♀     | Av.   |
| 25       | 14.63 | 0.45 | 0.50              | 0.90              | 0.160          | 13.42         | 2.628             | 1.886 | 2.257 |
| 29       | 14.63 | 0.67 | 0.33              | 2.03              | 0.099          | 9.04          | 0.693             | 1.488 | 1.091 |
| 32       | 16.19 | 0.45 | 0.50              | 0.90              | 0.057          | 13.13         | 1.062             | 0.719 | 0.891 |
| 22       | 9.75  | 0.67 | 0.33              | 2.03              | 0.071          | 8.41          | 1.312             | 0.459 | 0.885 |
| 31       | 12.87 | 0.45 | 0.50              | 0.90              | 0.080          | 9.91          | 0.689             | 0.750 | 0.719 |
| 38       | 16.19 | 0.22 | 0.22              | 1.00              | 0.056          | 9.32          | 1.197             | 0.146 | 0.671 |
| 37       | 12.87 | 0.22 | 0.22              | 1.00              | 0.067          | 8.87          | 0.923             | 0.315 | 0.619 |
| Belar    | 9.00  | 0.20 | 0.20              | 1.00              | 0.055          | 9.07          | 0.921             | 0.253 | 0.587 |
| 23       | 9.75  | 0.45 | 0.50              | 0.90              | 0.060          | 7.39          | 0.904             | 0.110 | 0.507 |
| 34       | 12.87 | 0.67 | 0.33              | 2.03              | 0.045          | 9.51          | 0.528             | 0.420 | 0.474 |
| 26       | 14.63 | 0.22 | 0.22              | 1.00              | 0.070          | 7.76          | 0.311             | 0.514 | 0.412 |
| 35       | 16.19 | 0.67 | 0.33              | 2.03              | 0.042          | 7.13          | 0.321             | 0.478 | 0.400 |
| 30       | 11.31 | 0.45 | 0.50              | 0.90              | 0.039          | 7.18          | 0.398             | 0.400 | 0.399 |
| 20       | 9.75  | 0.22 | 0.33              | 0.67              | 0.049          | 8.91          | 0.449             | 0.333 | 0.391 |
| Hobson   | 9.22  | 0.22 | 0.22              | 1.00              | 0.048          | 8.22          | 0.365             | 0.405 | 0.385 |
| 3        | 9.75  | 0.89 | 0.67              | 1.33              | 0.035          | 9.16          | 0.415             | 0.195 | 0.305 |
| 36       | 11.31 | 0.22 | 0.22              | 1.00              | 0.038          | 5.97          | 0.154             | 0.436 | 0.295 |
| Yeager   | 9.82  | 0.77 | 0.50              | 1.54              | 0.036          | 8.46          | 0.243             | 0.344 | 0.294 |
| 21       | 9.75  | 0.45 | 0.17              | 2.65              | 0.046          | 4.26          | 0.421             | 0.158 | 0.290 |
| 4        | 9.75  | 0.89 | 0.89              | 1.00              | 0.040          | 5.32          | 0.540             | 0.022 | 0.281 |
| 6        | 9.75  | 0.45 | 0.67              | 0.67              | 0.050          | 6.30          | 0.371             | 0.154 | 0.262 |
| 16       | 9.75  | 0.45 | 0.33              | 1.36              | 0.048          | 5.19          | 0.462             | 0.043 | 0.252 |
| 15       | 9.75  | 0.22 | 0.50              | 0.44              | 0.044          | 3.56          | 0.276             | 0.173 | 0.225 |
| 5        | 9.75  | 0.22 | 0.67              | 0.33              | 0.028          | 5.23          | 0.339             | 0.010 | 0.174 |
| 8        | 9.75  | 1.19 | 0.89              | 1.34              | 0.026          | 3.01          | 0.319             | 0.016 | 0.168 |
| 2        | 9.75  | 0.89 | 0.33              | 2.70              | 0.018          | 4.53          | 0.198             | 0.048 | 0.123 |
| 12       | 9.75  | 0.67 | 0.67              | 1.00              | 0.037          | 2.48          | 0.211             | 0.009 | 0.110 |
| TenCate  | 9.50  | 0.00 | 0.00              | 0.00              | 0.069          | 1.54          | 0.036             | 0.134 | 0.085 |
| 11       | 9.75  | 0.00 | 0.44              | 0.00              | 0.022          | 2.61          | 0.075             | 0.022 | 0.049 |
| Glaser   | 9.00  | 0.25 | 0.42              | 0.60              | 0.024          | 2.47          | 0.033             | 0.063 | 0.048 |
| 1        | 9.75  | 0.89 | 0.17              | 5.24              | 0.042          | 2.04          | 0.022             | 0.048 | 0.036 |
| 24       | 4.87  | 0.45 | 0.50              | 0.90              | 0.044          | 2.20          | 0.044             | 0.011 | 0.028 |
| 9        | 9.75  | 0.45 | 0.00              | 0.00              | 0.037          | 1.50          | 0.016             | 0.040 | 0.028 |
| 33       | 11.31 | 0.67 | 0.33              | 2.03              | 0.015          | 1.20          | 0.042             | 0.006 | 0.024 |
| 10       | 9.75  | 0.00 | 0.22              | 0.00              | 0.033          | 0.51          | 0.020             | 0.009 | 0.015 |
| 7        | 9.75  | 1.19 | 0.67              | 1.78              | 0.017          | 0.34          | 0.009             | 0.002 | 0.006 |
| 17       | 9.75  | 0.67 | 0.17              | 3.94              | 0.016          | 0.25          | 0.008             | 0.004 | 0.006 |
| 14       | 9.75  | 0.00 | 0.67              | 0.00              | 0.018          | 0.45          | 0.007             | 0.003 | 0.005 |
| 13       | 9.75  | 0.00 | 0.89              | 0.00              | 0.037          | 0.17          | 0.002             | 0.009 | 0.005 |
| 28       | 4.87  | 0.67 | 0.33              | 2.03              | 0.009          | 0.37          | 0.003             | 0.004 | 0.004 |
| 27       | 4.87  | 0.22 | 0.22              | 1.00              | 0.004          | 0.69          | 0.005             | 0.002 | 0.004 |
| 18       | 9.75  | 0.89 | 0.00              | 0.00              | 0.006          | 0.68          | 0.001             | 0.004 | 0.003 |
| 19       | 9.75  | 1.19 | 0.00              | 0.00              | 0.009          | 0.11          | 0.003             | 0.001 | 0.002 |

\*Figures for amplitude of contraction (Ampl.) and for hours of activity (Time) are averages based on tracings from 10 crops tested in each solution.

the highest average activity product, and maintained contractions, in one instance, for more than 26 hours. Although several exceptions were present, it appeared also that, in general, the concentrations of both KCl

and  $\text{CaCl}_2$  should be less than 1.0 gram per liter of solution and that the  $\text{KCl}/\text{CaCl}_2$  ratio must be within the range of 0.7 to 2.0. Investigations to determine optimum concentrations for these salts are now in progress.

When the various  $\text{NaCl}$  percentages were tested with the three  $\text{KCl}-\text{CaCl}_2$  concentrations (see methods for details), the following results were obtained. With only 4.87 grams of  $\text{NaCl}$ , the activity products dropped to almost nothing, as with solutions 24, 27, and 28 (0.028, 0.004, 0.004). Apparently the tissue could not maintain contractions with this lowered content of  $\text{NaCl}$ . However, when the amount of  $\text{NaCl}$  was increased, greater activity resulted. Ranked according to activity products, nine of the first twelve solutions had  $\text{NaCl}$  concentrations higher than 12.87 grams per liter. Six of these nine maintained an average activity time of more than nine hours (see table 2). Not only was the length of time of activity longer when the  $\text{NaCl}$  was increased, but also the average amplitude was higher. Among the high  $\text{NaCl}$  concentrations are average amplitude readings of 0.160, 0.099, and 0.080 centimeter. The lower  $\text{NaCl}$  levels approached these values in only one instance, number 22, with 9.75 grams per liter which gave an average amplitude of 0.71 centimeter.

Plate I consists of kymograph records which indicate the difference in general appearance between tracings made by crops in various salt mixtures. *A* and *F* were made in solution 25 which has given the best results (average activity product of 2.3) in tests so far conducted; *B*, *C*, *D*, and *E* are recordings of crop activity in solutions 21, 20, 23, and 20, respectively. These latter salt mixtures gave average activity products approximately one-fourth to one-seventh of that of number 25. Poorer solutions would give records with only a few erratic contractions. Figure 2 also demonstrates that baselines in records from certain solutions are quite irregular, as in *B*, *C*, *D*, and *E*. This variability was particularly evident during the first hour or hour and a half of a crop's subjection to solutions with less than 10 grams of  $\text{NaCl}$  per liter, which was the case in the mixtures from which these records resulted. Often many minutes passed before contractions started. In general, activity in the solutions with high  $\text{NaCl}$  (12.87 grams or more) started immediately with high amplitude and a stable baseline, (*A* and *F*), both of which continued throughout the major portion of the time of activity. These conditions were especially evident in solution number 25, which contains 14.63 grams of  $\text{NaCl}$ , 0.45 grams of  $\text{KCl}$ , 0.50 gram of  $\text{CaCl}_2$ , and 0.19 grams of  $\text{NaHCO}_3$ . (Plate I.)

A more thorough investigation of higher levels of  $\text{NaCl}$ , with other  $\text{KCl}-\text{CaCl}_2$  concentrations, are now in progress to ascertain a mixture of optimum salt concentrations for the type of experiment described. The results from the above trials seem to indicate that greater amounts of  $\text{NaCl}$ , than those which have been reported in the literature (see table 1), are beneficial in maintaining high amplitude and longevity of muscular contraction in the fore-gut of the American roach. Whether the possibility that such a solution will be good for all types of roach tissue, or for

materials from other insect species, has not yet been explored. It is worthy of note, however, that Yeager (13), in 1939, found that increasing the NaCl concentrations in his physiological solutions to 10.93 and 11.78 grams per liter gave better results when studying electrical stimulation of the dorsal vessel in the cockroach, *Periplaneta americana*. Previously he had used 9.0 or 9.82 grams of NaCl per 1000 cc.

The supposed role of the sodium ion is that of an osmotic regulator and a stimulatory ion. Cole and co-workers (3) found, while investigating heart contractions in the crayfish, that high concentrations of the sodium ion alone stimulated the heart to greater frequency, but proved to be toxic in that the longevity of the tissue was materially reduced. Also, according to Rogers (8, pp. 156), "it (sodium) exerts in many cases a distinct poisonous effect, acting particularly upon the cell membranes." Thus it appears that although an increase in the sodium content may cause hyperactivity, this increased response may last for only a short time, and over long periods of exposure, high sodium ion concentrations are detrimental. It seems, therefore, that a higher level of NaCl may be beneficial in producing an increased activity if it is maintained below a point where the ion exerts a toxic effect; and it appears from the foregoing experiments that a higher NaCl concentration than has so far been recommended in insect salines does have definite merit in maintaining the activity of the isolated fore-gut of the roach.

During the course of the present investigations a distinct difference was observed in both the appearance and the activity of the crops from male and female roaches. When analyzed statistically, there was a significant difference in the amount of food present in the crop. The crops from males were definitely more active and had food present in only a small number of cases. The actual presence of food, however, did not seem to be correlated with hyper- or hypo-activity of the crop. No attempt has been made to regulate the period of feeding prior to experimentation, but both males and females had equal access to the same kind of food. At present no adequate explanation can be advanced. However, Snipes and Tauber (10) found that, while not significantly different, the egestion time of female specimens of *Periplaneta americana* was slower than that of males (19.6 hours for males; 21.4 for females). Therefore, it appears that the muscular activity of the crop and digestive tract in the male American roach is greater than that of the female.

#### SUMMARY

1. A method for recording and measuring the muscular activity of the excised fore-gut from the American roach is described. See figure 1.
2. Formulae for insect physiological salines which have been published were tested and found not to maintain activity as well as some other solutions which were devised during the course of the present experiments. See table 1. Published formulae gave activity products



which ranged from 0.048 to 0.587; the best solution tested in the present series by the writers gave an average activity product of 2.257.

3. Several concentrations of KCl (0.20 to 0.89 gram per liter) and of  $\text{CaCl}_2$  (0.20 to 0.67 gram per liter) will maintain contractions of the excised crop, over a period averaging from 9 to 13 hours, when combined with the correct amount of NaCl, usually from about 10 to 15 grams per liter. The maximum length of activity so far obtained from any single crop was over 26 hours in solution number 22 which contained 9.75 grams of NaCl. (In general, however, results from 22 were not as good as from other solutions with higher NaCl content.)

4. Sodium chloride in concentrations of 12.87 to 16.19 grams per liter of solution, when combined with certain KCl- $\text{CaCl}_2$  ratios, appears to be beneficial by increasing the amplitude of contraction and the longevity of the excised tissue.

5. Solution 25 (14.63 grams of NaCl, 0.45 gram of KCl, 0.50 gram of  $\text{CaCl}_2$ , and 0.19 gram of  $\text{NaHCO}_3$  per liter) produced an activity product twice as high as the next best solution and maintained crop activity for an average of more than 13 hours. Minimum duration was 3.7 hours; maximum, 25.3 hours. This mixture has not been tested on other tissues or under other conditions.

6. The crops from male roaches produced significantly higher activity products than those from females. Food was present in the fore-gut of the females more often than in the males.

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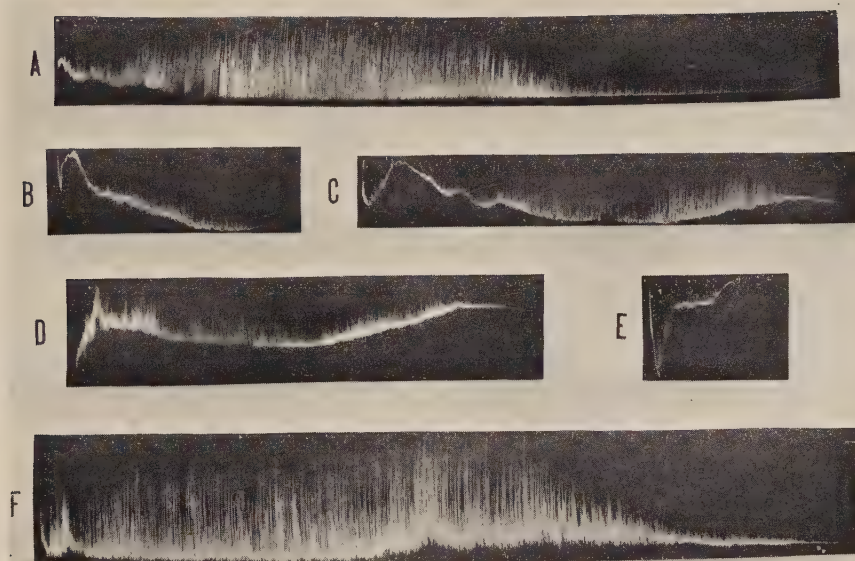


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#### PLATE I

Kymograph records of isolated crop activity. *A* and *F* were made in the best solution so far devised (number 25). *B*, *C*, *D*, and *E* are records from poorer solutions (number 21, 20, 23, and 20, respectively) and show the irregular baseline, erratic muscular response, and delayed beginning of contractions produced in such solutions. (See Table 2 for composition and activity products of these salt mixtures.)

PLATE I





# EFFECT OF ETHER ON THE TOXICITY OF CERTAIN FUMIGANTS TO THE CONFUSED FLOUR BEETLE, *TRIBOLIUM* *CONFUSUM* DUVAL<sup>1</sup>

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Received June 14, 1940

The study of synergism and antagonism in fumigant mixtures is a new and little-known field although these effects have been more thoroughly investigated in the field of local and general anesthesia. Synergism is defined broadly as "cooperation among several component parts" and in a toxicological sense may be said to be a lethal effect greater than additive. Antagonism, conversely, is a lethal effect less than additive.

The mechanism through which synergism or antagonism is obtained is not thoroughly understood. Bancroft and Richter (1931) explain synergistic action as that following the displacement of a drug by another drug which is adsorbed onto the same substrate. This displacement in effect increases the active or effective concentration of the primary drug and thus increases its toxicity in the cell. Bancroft and Richter (1931) state that antagonism occurs when the colloids of a cell are reversibly coagulated and the coagulating agent is replaced by a substance of weaker flocculation capacity at the given concentration. In this event the biocolloids are again peptized by the electrolytes of the cell.

The toxicity of ether to various insects has been investigated by a number of workers. McClintock, Hamilton and Lowe (1911) reported that carbon disulfide was apparently four times as toxic as ether to the housefly (*Musca domestica* L.), and that ether and chloroform were equal in their effect on houseflies. Shafer (1915) found that ether absorbed by an adult beetle (*Passalus cornutus*) did not interfere with the activity of the catalase present. Holt (1916) showed that carbon disulfide is about four times as toxic as ether to the cockroach. Moore (1917) states that carbon disulfide is roughly fifteen times as toxic as ether to houseflies. He found also that carbon tetrachloride was roughly twenty-seven times as toxic as ether to this insect.

Many other investigators have studied the effects of ether on insects. Among these are Moore and Graham (1918), Roark and Cotton (1929) and others. All have agreed that ether possesses low toxicity toward insects.

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<sup>1</sup> Journal Paper No. J-767 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 137.

<sup>2</sup> The writer gratefully acknowledges the inspiration and guidance of Dr. C. H. Richardson during the course of this research.



Carbon disulfide has been used as an insect fumigant since 1854, according to Simmons and Ellington (1926).

Carbon tetrachloride has been used as a fumigant only since about 1907, when Britton (1907, 1908a, 1908b) investigated the possibilities of this compound as a fumigant for nursery stock. Both of these compounds have been thoroughly tested by many competent workers on a wide variety of insects.

Ethyl acetate was recommended as a fumigant in combination with carbon tetrachloride by Back and Cotton (1925). It was later found that this material, although relatively non-inflammable, left an objectionable odor in fumigated grain. Shepard, Lindgren and Thomas (1937) state that a non-inflammable mixture of ethyl acetate and carbon tetrachloride is of low toxicity to stored-grain insects.

Neifert, et al. (1925) used a number of gas mixtures against adults of some of the more important grain-infesting insects. The authors state that less toxic substances in these mixtures were used principally as diluents.

Hazelhoff's (1928) experiments led him to make the suggestion that the addition of carbon dioxide to hydrogen cyanide, carbon disulfide and other fumigants would increase the toxicity of these compounds by increasing the rate of penetration of the gas.

Cotton and Roark (1927, 1928) and Roark and Cotton (1928) tested a number of gas mixtures and recommended a mixture containing three volumes of ethylene dichloride and one volume of carbon tetrachloride. This mixture was non-burnable and non-explosive.

Cotton and Young (1929) and Cotton (1930) showed that carbon dioxide increased the toxicity of given concentrations of carbon disulfide and chloropicrin to a marked degree. Back, Cotton and Ellington (1930) found that the addition of carbon dioxide greatly increased the efficiency of ethylene oxide as a fumigant.

Cotton (1932) found that carbon dioxide gave a maximum increase in toxicity to adults of *T. confusum* at concentrations of 37 lb. per 1000 cu. ft. with chloropicrin, 15 lb. per 1000 cu. ft. with carbon disulfide and 30 lb. per 1000 cu. ft. with ethylene oxide. When more than these amounts of carbon dioxide were added, the toxicity decreased.

Shepard and Lindgren (1934) give data on the relative toxicities of ethylene dichloride, propylene dichloride and their 75 per cent mixtures with carbon tetrachloride which show definite antagonism between these compounds and carbon tetrachloride.

Cupples, Yust and Hiley (1936) placed ether-HCN and carbon tetrachloride-HCN in the group of mixtures showing slight or no toxicity to red scale, while a carbon disulfide-HCN mixture was moderately toxic.

Cotton (1938) recommends the use of mixed gases for the control of insects attacking grain in farm storage. He states that mixtures of carbon disulfide with carbon tetrachloride and sulfur dioxide, to reduce the fire hazard, are now commercially available.

## EXPERIMENTAL METHOD

The insects used in these experiments were adults of the confused flour beetle (*Tribolium confusum* Duval). They were reared at 30° C. and a relative humidity of 60-70 per cent on white flour to which dried powdered yeast was added. Only beetles between 2 and 6 weeks of age were used in tests. During these experiments none of the many unfumigated check insects died, so it is apparent that natural mortality is a negligible factor within this age group.

The fumigation chambers employed in these experiments were balloon flasks of approximately 5.5 liters capacity. The apparatus was slightly modified from that used by Jones (1933) since all of the fumigants were introduced as liquids by means of burettes graduated to 0.01 cc.

From 30 to 50 adult beetles, selected at random from a large group, were used in each experiment. These were placed in glass cylinders, open at the top and closed at the bottom by one layer of cheesecloth, after which the cylinders were suspended from hooks in the tops of the flasks. The flasks were partially evacuated and the liquid fumigant introduced. The flasks were then placed at  $30^{\circ} \pm 0.5^{\circ}\text{C.}$  and returned to atmospheric pressure as soon as the liquid had evaporated. The beetles were exposed to the fumigant for a period of 2 hours, after which they were placed on clean flour at 30°C.

The temperature of 30°C. was used in order that the gases might be employed at higher activities than at 25°C. The exposure time of 2 hours was selected solely because of convenience, since a greater amount of data could be obtained in a given length of time.

Mortality counts were made at the end of 24, 48 and 96 hours. As stated by Shepard, Lindgren and Thomas (1937) and shown by Hamlin and Reed (1927, 1928) a serious source of variation lies in the difficulty of determining a sharp endpoint. In these experiments the final counts were made at the end of 96 hours. At that time the beetles were placed in one of three categories: alive, paralyzed in one or more pairs of legs, and dead. After exposure to ether and carbon disulfide, the beetles so injured by the fumigant that they were paralyzed at the end of 96 hours generally failed to recover. When these fumigants were employed, both paralyzed and dead beetles were used in determining the total mortality. With carbon tetrachloride, however, it was found that beetles completely paralyzed in as many as two pairs of legs might live for as long as a month in that condition and produce eggs. Here, only the number of beetles actually dead was used in determining the total mortality. The end point with ethyl acetate was quite sharp, and there were only minor changes in mortality after the 24-hour count. No great amount of paralysis was noted from this compound and only the number actually dead was used in determining the final mortality at 96 hours.

The data obtained from these experiments were plotted and analyzed statistically according to the method given by Bliss (1935). By using this method it is possible to transform the usual sigmoid dosage-mortality curve into a straight-line regression. The chi-square test may be applied to this regression line in order to determine the goodness-of-fit of the data. In addition, the limits of error may be calculated such that 95 per cent of the observations should fall within given areas or zones along the line. These limits of error were used in determining the antagonistic or synergistic effect of added toxic substances.

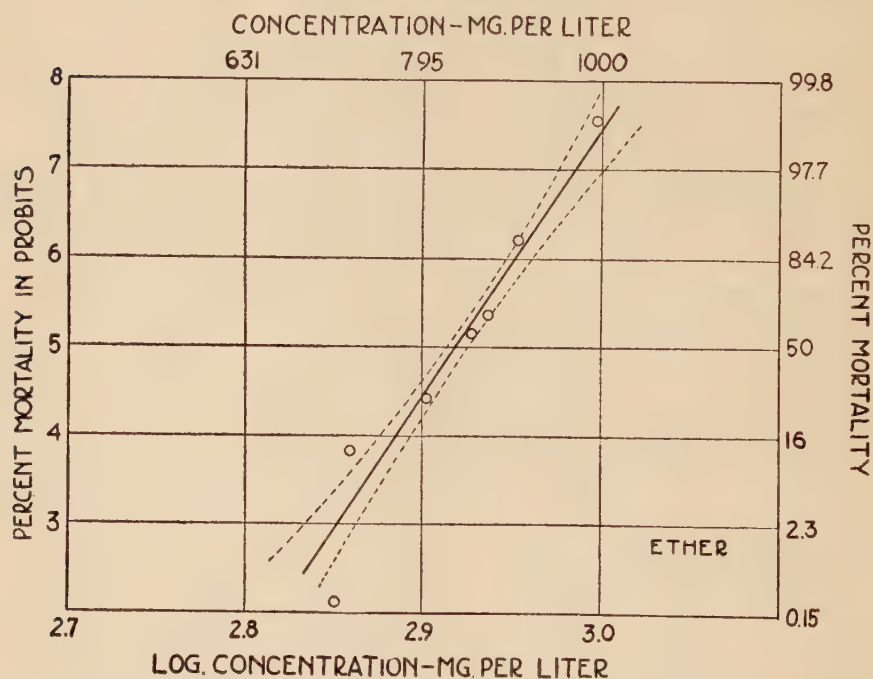


FIG. 1. Toxicity of ether to *Tribolium confusum* Duval.

The first series of experiments was concerned with the determination of the 2-hour median lethal concentrations of ether, carbon disulfide, carbon tetrachloride and ethyl acetate. Since the dosage-mortality relationship of ether to *Tribolium confusum* has not been reported previously, this curve was established rather carefully through a large number of experiments. Many previous workers have determined the effects of carbon disulfide, carbon tetrachloride and ethyl acetate on the confused flour beetle at a 5-hour exposure and 25°C. Shepard, Lindgren and Thomas (1937) also record the concentration of carbon disulfide and car-



bon tetrachloride killing 50 per cent and 99 per cent in 5 hours at 30°C. For that reason less time was spent in the determination of these curves.

Following these tests, the effect of the addition of sublethal concentrations of ether to the median lethal concentration of each of the other gases was determined. It was felt that any synergistic or antagonistic effect of ether upon the other gases would appear more strongly under such conditions.

## RESULTS

### A. TOXICITY OF FUMIGANTS ALONE

The 2-hour dosage-mortality curves at 30°C. for ether, carbon disulfide, carbon tetrachloride and ethyl acetate are shown in figures 1, 2, 3, and 4. The 2-hour median lethal concentrations and the concentrations killing 99 per cent of the insects are given in table 1.

TABLE 1. Median lethal concentrations of fumigants against *Tribolium confusum* Duval

| Fumigant             | No. of insects tested | Concentration, in mg. per liter, required to kill— |              |
|----------------------|-----------------------|--|--------------|
|                      |                       | 50 per cent  | 99 per cent* |
| Ether                | 5800                  | 832  | 991          |
| Carbon disulfide     | 1150                  | 115  | 178          |
| Carbon tetrachloride | 1287                  | 135  | 248          |
| Ethyl acetate        | 750                   | 108  | 148          |

\* Calculated from Bliss' formula  $Y=a+b(X-x)$ .

The chi-square test was applied to each of the calculated dosage-mortality curves in order to determine the homogeneity of the data. The chi-square calculated for the ether curve showed that more variation was present than is expected from random sampling of a homogeneous population. The chi-square test applied to the carbon disulfide curve (fig. 2) indicated strongly that these data are homogeneous and any variation present may be expected from random sampling. The data used in the determination of the carbon tetrachloride curve (fig. 3) show heterogeneity when analyzed by means of the chi-square test. The results of fumigation with ethyl acetate are homogeneous as shown by the chi-square test.

The presence of heterogeneity in the dosage-mortality curves for ether and carbon tetrachloride may be due to the fact that these two compounds cause a rather confusing paralysis making determination of the end point difficult in some of the insects exposed in the tests. As discussed in a previous section, ethyl acetate and carbon disulfide are more definite in action than are ether and carbon tetrachloride.



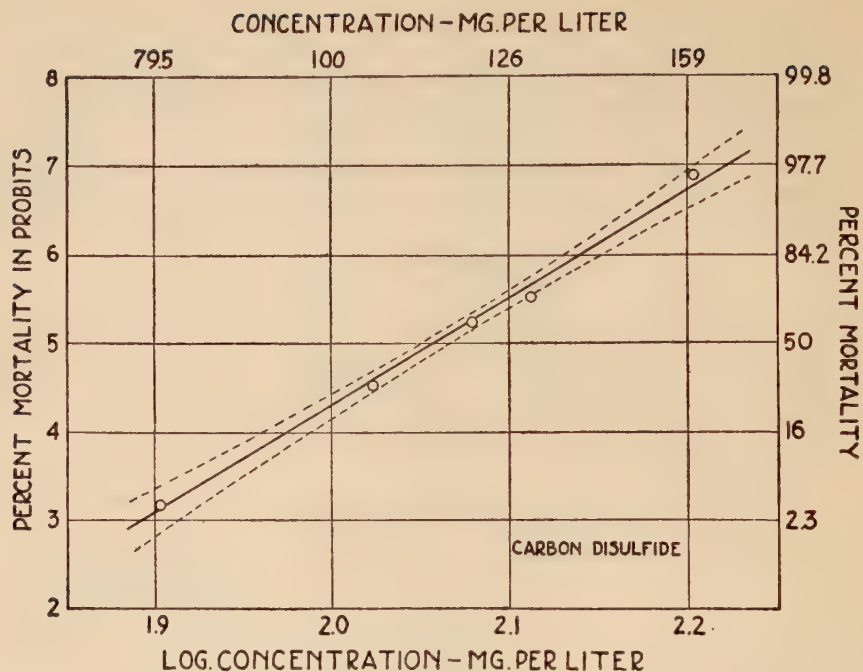


FIG. 2. Toxicity of carbon disulfide to *Tribolium confusum* Duval.

#### B. TOXICITY OF FUMIGANTS WITH SUBLETHAL CONCENTRATIONS OF ETHER

Following the experiments reported in the preceding section, it was thought desirable to determine the effect of sublethal concentrations of ether on the median lethal concentrations of the other gases.

The concentrations of ether to be used were determined in the following manner: small amounts of ether, ranging from 33 mg. per liter to 600 mg. per liter were placed in flasks containing flour beetles. Observations were made at frequent intervals and the concentrations used here were selected on the basis of the reaction of the insects. It was found that 33 mg. per liter did not anesthetize the beetles during the 2-hour exposure; 130 mg. per liter caused anesthesia in 45 to 60 minutes; and 520 mg. per liter anesthetized in less than 5 minutes after exposure. None of these concentrations killed any of the insects.

These concentrations (33, 130, 520 mg. per liter) were used with the approximate median lethal concentration of each of the other gases. It was thought that the primary effect of ether on the toxicity of the other gases might be due to its known anesthetic ability. If this were true there would be a consistent decrease in toxicity from 520 to 33 mg. per liter in the ether-fumigant mixture. The results of these tests are shown in table 2 and in figures 5, 6 and 7.

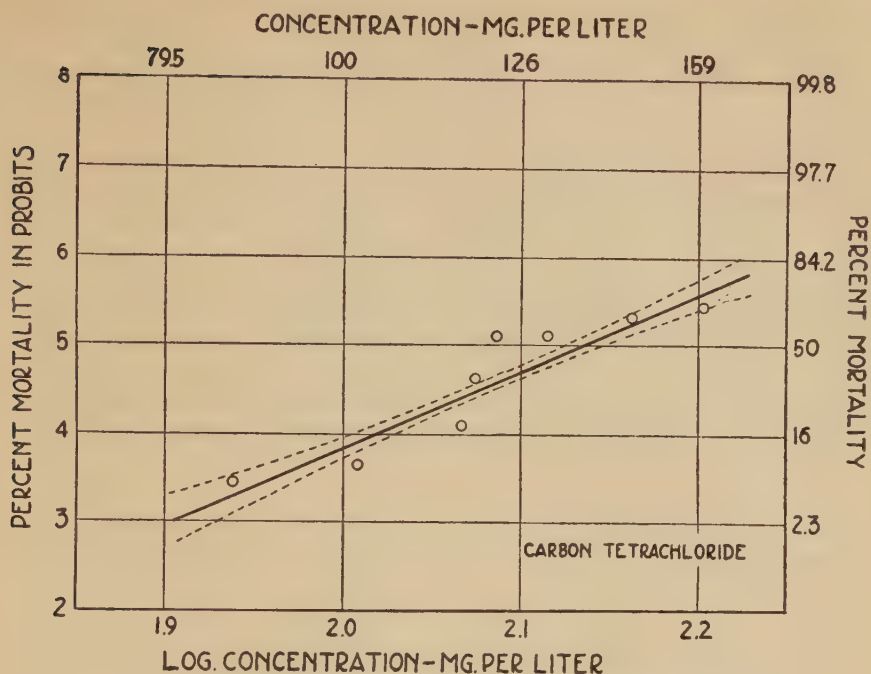


FIG. 3. Toxicity of carbon tetrachloride to *Tribolium confusum* Duval

The chi-square test as given by Snedecor (1937) was applied to these data. In table 2, the figure marked with an asterisk is the average of tests in which the variation was greater than that expected from random sampling. It is shown, however, that there is a definite trend through the regions of these points.

#### DISCUSSION

The relative toxicity of ether, carbon disulfide, carbon tetrachloride and ethyl acetate to the confused flour beetle is shown in table 1. The median lethal concentrations are roughly in the ratio 7.7:1.0:1.2:1.0, indicating that carbon disulfide, carbon tetrachloride and ethyl acetate are all about seven times as toxic to *Tribolium confusum* as ether at an exposure of 2 hours at 30°C. It is interesting to note the rather small degree of variation at 50 per cent mortality shown by carbon disulfide, carbon tetrachloride and ethyl acetate, although these compounds differ widely from one another in chemical composition.

At the concentrations calculated to cause 99 per cent mortality, the relationship changes and the ratio ether: carbon disulfide: carbon tetrachloride: ethyl acetate is 6.7:1.2:1.7:1.0, indicating that at the higher level of mortality carbon disulfide, carbon tetrachloride and ethyl acetate

are, in general, less than six times as toxic to *Tribolium confusum* as ether under these experimental conditions.

Shepard, Lindgren and Thomas (1937) record the 5-hour median lethal concentrations at 30°C. for carbon disulfide (44 mg. per liter) and carbon tetrachloride (125 mg. per liter). It may be seen from these figures that at 5 hours and 30°C. the ratio carbon disulfide to carbon tetrachloride is 1:2.8, while at 2 hours and 30°C. this ratio is about 1:1.1. This seems to indicate that with carbon disulfide toxicity increases more rapidly with an increase in time of exposure than with an increase in temperature, whereas the toxicity of carbon tetrachloride increases more rapidly with a rise in temperature than with an increased exposure time.

These authors also include the concentrations of carbon disulfide and carbon tetrachloride calculated to kill 99 per cent of the test beetles. When the concentrations of carbon disulfide causing 50 per cent mortality at exposure times of 2 and 5 hours are compared, it is seen that the ratio

TABLE 2. Toxicity of fumigants with sublethal concentrations of ether

| Concentration in mg. per liter |                         | Expected<br>Mortality<br>Limits | Percentage<br>Mortality<br>Obtained |
|--------------------------------|-------------------------|---------------------------------|-------------------------------------|
| Ether                          | Carbon<br>disulfide     |                                 |                                     |
| ...                            | 115                     | 45.5 to 53.9                    | 50.0                                |
| 33                             | 115                     | 45.5 to 53.9                    | 82.2                                |
| 131                            | 115                     | 45.5 to 53.9                    | 97.3                                |
| 522                            | 115                     | 45.5 to 53.9                    | 100.0                               |
| Ether                          | Carbon<br>tetrachloride | 41.1 to 47.2                    |                                     |
| ...                            | 130                     |                                 |                                     |
| 33                             | 130                     |                                 |                                     |
| 130                            | 131                     |                                 |                                     |
| 521                            | 130                     |                                 |                                     |
| Ether                          | Ethyl acetate           | 43.7 to 56.3                    |                                     |
| ...                            | 107                     |                                 |                                     |
| 33                             | 107                     |                                 |                                     |
| 130                            | 107                     |                                 |                                     |
| 522                            | 107                     |                                 |                                     |

5:2 hours exposure is 1:2.61 and at 99 per cent mortality, the ratio 5:2 hours is also 1:2.61. This would indicate that the curves for carbon disulfide at 5 hours and 2 hours at a temperature of 30°C. are nearly parallel throughout, since the ratios at the 50:99 per cent mortality are identical.

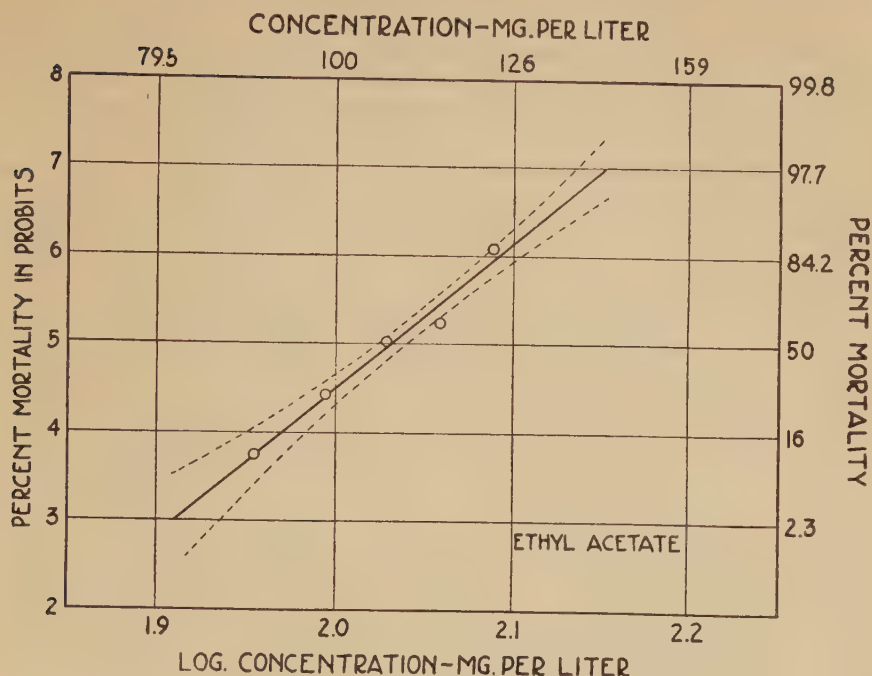


FIG. 4. Toxicity of ethyl acetate to *Tribolium confusum* Duval.

A comparison of the ratio 5:2 hours exposure of carbon tetrachloride at the 50 per cent point shows that it is about 1:1.08, while at the 99 per cent point the ratio 5:2 hours exposure is 1.97:1, indicating that the 5-hour dosage-mortality curve of carbon tetrachloride crosses the 2-hour curve at a point somewhere above the median lethal concentration.

This same phenomenon occurs in the carbon tetrachloride curves at 25°C and 30°C. where a 5-hour exposure was used, according to Shepard, Lindgren and Thomas (1937). The concentration of carbon tetrachloride killing 99 per cent at 25°C. is 405 mg. per liter, while that at 30°C. is 490 mg. per liter. It would appear that this effect of carbon tetrachloride is present over a wide range of temperature and exposures.

Mixtures containing the median lethal concentration of carbon disulfide, carbon tetrachloride or ethyl acetate with sublethal concentrations of ether show considerable differences in toxicity to the confused flour beetle. It is apparent from an inspection of figures 5 and 6 that carbon disulfide and carbon tetrachloride are similar in their actions when combined with sublethal concentrations of ether. Mixtures of both compound are least toxic to the confused flour beetle at an ether concentration of 33 mg. per liter. At higher ether concentrations the toxicity increases rapidly and approaches 100 per cent mortality. When the median lethal concentration of ethyl acetate is combined with sublethal concentrations of ether, the toxicity of the mixtures varies greatly. Figure 7



shows that the mixture containing 107 mg. per liter of ethyl acetate and 33 mg. per liter of ether is definitely synergistic, while mixtures containing greater amounts of ether show decreasing toxicity, and at an ether concentration of 521 mg. per liter, probable antagonism is shown.

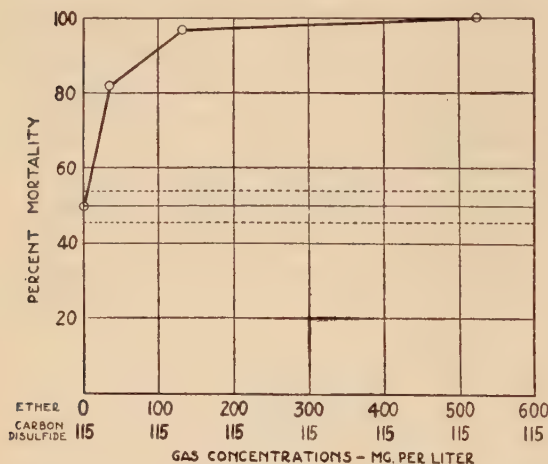


FIG. 5. Toxicity of carbon disulfide with sublethal concentrations of ether.

In a previous section, the action on the flour beetle of the sublethal ether concentrations used in these tests was described. Carbon disulfide appears to anesthetize or inactivate rather quickly insects exposed to this gas, while carbon tetrachloride inactivates test insects slowly and incompletely at low concentrations and more rapidly and completely at concentrations causing mortalities higher than 50 per cent. Ethyl acetate completely inactivates the confused flour

beetle only at concentrations causing mortalities above 50 per cent.

These experiments indicate that carbon disulfide and carbon tetrachloride are more toxic to inactivated flour beetles, while ethyl acetate is more toxic (within limits) to active insects. It is possible that when sublethal concentrations of ether are added to the median lethal concentration of carbon disulfide or carbon tetrachloride, the ether vapor, being lighter and perhaps penetrating more rapidly than the vapor of the other compound, attacks the insects at once. This attack may

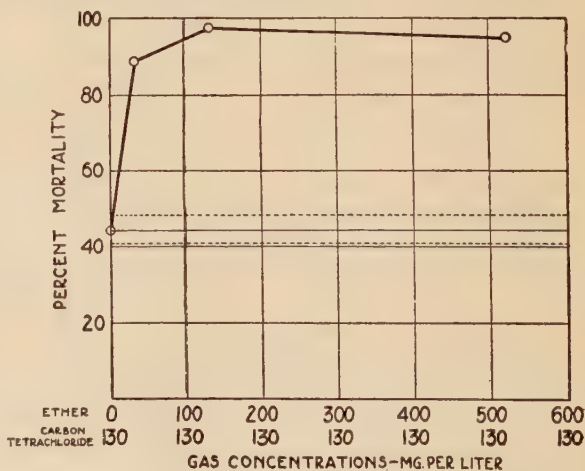


FIG. 6. Toxicity of carbon tetrachloride with sublethal concentrations of ether.

be directed upon the cells of the nervous system or against some other system. Following this, the heavier gas may enter the body more freely

and might be actually allowed a longer period of exposure than when acting alone. It is suggested, however, that ethyl acetate may exert its toxic action to the greatest extent upon active beetles, possibly because of poorer penetrating ability, and that anesthetizing sublethal concentrations

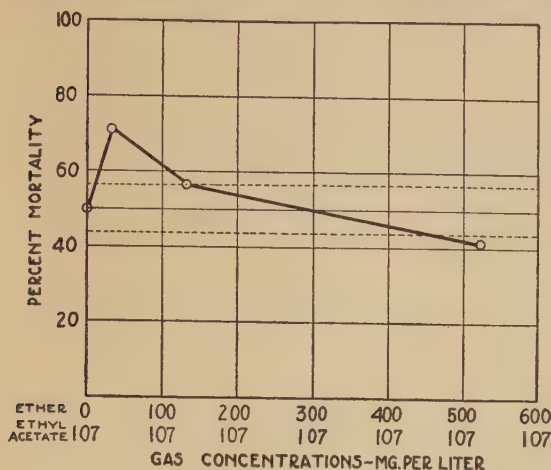


FIG. 7. Toxicity of ethyl acetate with sublethal concentrations of ether.

of ether cause a "protective stupefaction." The existence of this condition was first brought out by Gray and Kirkpatrick (1929) in experiments on HCN-resistant black and red scales.

#### SUMMARY AND CONCLUSIONS

The 2-hour dosage-mortality curves of ether, carbon disulfide, carbon tetrachloride and ethyl acetate at 30° C. against the confused flour beetle (*Tribolium confusum* Duval) are presented. Carbon

disulfide, carbon tetrachloride and ethyl acetate are more than seven times as toxic to the flour beetle as ether at the median lethal concentration under these experimental conditions.

The effect of certain sublethal concentrations of ether upon the toxicity of the median lethal concentrations of carbon disulfide, carbon tetrachloride and ethyl acetate was studied. It is shown that the toxicity of carbon disulfide and carbon tetrachloride is increased as the concentration of ether increases. The toxicity of ethyl acetate to the confused flour beetle decreases rapidly as the concentration of ether increases, and it was found that the highest sublethal concentration of ether used was apparently antagonistic with ethyl acetate.

Sublethal concentrations of ether increase the toxicity of carbon tetrachloride and carbon disulfide in proportion to the concentration of ether present. Sublethal concentrations of ether decrease the toxicity of ethyl acetate in proportion to the concentration of ether in the mixture.

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## AUTHOR INDEX

- Abbott, Clement Wilfred, 3  
 Aikman, J. M., 385  
 Anderson, A. A., 187  
 Atanasoff, J. V., 333
- Babel, Fred John, 6  
 Bailie, James Clyde, 8  
 Bartlett, John Bruen, 11  
 Becker, Elery R., 317  
 Brewer, Carl Robert, 14  
 Brewer, H. E., 385
- Clare, Stewart, 107  
 Claydon, Thomas Joseph, 17
- Davis, Arthur W., 20  
 Decker, G. C., 345  
 Deonier, Christian Charles, 22  
 Dinger, J. E., 195  
 Drake, C. J., 345  
 Dunning, John W., 24  
 Dykstra, Kenneth G., 27  
 Dyme, Harry Chaim, 29
- Ellisor, L. O., 305
- Fouts, Everett L., 32
- Gaessler, W. G., 379  
 Geiger, Charles, 367  
 Gilman, Joseph C., 129  
 Goodwin, Ralph Abijah, 35  
 Griffiths, J. T., Jr., 393  
 Gross, George Lloyd, 37  
 Gunderson, Harold, 39, 405  
 Guymon, James F., 40
- Haber, E. S., 379  
 Hammer, B. W., 163  
 Harned, Horace H., 43  
 Harris, Halbert M., 323  
 Hart, Phillip James, 46  
 Hayden, Ada, 199  
 Hixon, R. M., 379  
 Hodgdon, Frank B., 48  
 Holl, D. L., 295
- James, Norman, 50
- Kirchner, Justus G., 53  
 Kunerth, William, 195
- Landee, Franc A., 55  
 Leffert, Ila, 155  
 Levine, Max, 233  
 Lichtenwalter, Myrl, 57  
 Lineken, Edgar Elwyn, 60  
 Long, H. F., 163
- McCorkle, Miles R., 64  
 McCoy, D. Oren, 67  
 McNew, G. L., 129
- Mavis, H. C., 155  
 Mickelson, M. N., 253
- Nelson, G. H., 233  
 Nelson, M. E., 359
- Oldham, Wilbur, N., 69
- Richardson, Charles H., 305  
 Robertson, Fred, 261  
 Rock, Donald Hill, 71  
 Rosenbusch, Carlos T., 73  
 Russell, Morell B., 76
- Schoene, Lorin, 78  
 Silverman, M., 179  
 Simmons, Samuel William, 81  
 Spain, Louis A., 353  
 Stebbins, Dean Waldo, 84  
 Stone, R. W., 253  
 Straka, R. P., 233  
 Sullivan, John L., 87  
 Sutton, William R., 89  
 Swislow, Jack, 92
- Tate, H. D., 267  
 Tauber, Oscar E., 107, 393  
 Thorne, Alison Comish, 95  
 Thorne, C. J., 333  
 Tripp, R. H., 295  
 Turck, Joseph A. V., Jr., 98
- Utter, M. F., 179
- Van Peursem, Ralph Lawrence, 101
- Werkman, C. H., 179, 187, 253, 359, 367  
 Whisler, B. A., 215  
 Wiggert, W. P., 179  
 Wood, H. G., 367  
 Wyatt, Waldo Richard, 103

## SUBJECT INDEX

- Accuracy of the plating method for estimating the number of bacteria, actinomyces and fungi in a laboratory sample of soil, The, 50
- Acetobacter suboxydans*, oxidation of polyhydric alcohols by, 24
- Achromobacter putrefaciens*, relationship of, to putrid type of cheesiness in butter, 17
- Acid, citric, bacterial dissimilation of, 14
- Acid forming organism, description of, 187
- Acid, phosphoglyceric, 253
- Actinomyces, estimating number of, in soil, 50
- Active juice, preparation of, from bacteria, 179
- Aerobacter indologenes*, 253
- Aerobacter indologenes*, active juices from, 180
- Alcohols, polyhydric, oxidation of, by *Acetobacter suboxydans*, 24
- Alternating voltages, 67
- Amino acids, use of, in study of heterofermentative lactic acid bacteria, 377
- Anaerobic decomposition and gasification of cornstalks by thermophiles, 233
- Angiospermae, 199
- Antibody response to experimental swine influenza, 73
- Aphids, as vectors of virus disease of onions, 275
- Apioporthes, and their related forms, 129
- Azotobacter, factors influencing nitrogen fixation by, 87
- Bacillus*, dextro-lactic acid forming organism of, 187
- Bacteria, estimating number in soil, 50
- Bacteria, heterofermentative lactic acid, 353
- Bacteria, heterofermentative lactic acid, nutritive requirements of, 367
- Bacteria, preparation of active juices from, 179
- Bacteria, use of ultra-violet light in killing, 215
- Bacterial dissimilation of citric acid, 14
- Bacteriological studies on Swiss-type cheese from pasteurized milk, 6
- Beetle, confused flour, effect of ether on toxicity of certain fumigants to, 39
- Beetle, confused flour, toxicity studies on, 405
- Beryllium salts, 89
- Betulaceae (Birch family), 205
- Blattella germanica* L. (cockroach), circulation of hemolymph in wings of, 107
- Bordeaux spray, 155
- Breakdown potentials of gases under alternating voltages, 67
- Burri smear culture technic, 163
- Butter, cultures of, effects of tomato juice on flavor, 3
- Butter, putrid defect of, relationship of *Achromobacter putrefaciens* to, 17
- Butyl-acetonic fermentation, 40
- Cadmium salts, 89
- Cankers, tree, in Iowa, fungi associated with, 129
- Caprifoliaceae (Honeysuckle family), 211
- Castor bean plants, reaction of grasshoppers to, 353
- Ceramic kilns, radiant heat transfer in, 48
- Cereals, relation of saccharification and yield of ethanol from, 78
- Cheese, Swiss-type, bacteriological studies on, 6
- Chemoreceptors, gustatory, effects of toxic compounds on, in Diptera, 22
- Cholesterilene, preparation of, 101
- Circulation of hemolymph in the wings of the cockroach, *Blattella germanica* L. I. In normal wings, 107
- Citric acid, bacterial dissimilation of, 14
- Citrobacter freundii*, preparation of active juices from, 186
- Classification of the proteolytic micrococci isolated from dairy products, A, 43
- Clostridium butylicum*, preparation of active juices from, 186
- Cockroach (*Blattella germanica*), normal wings of, 107
- Comparative value of sprays and dusts in insect control, 155
- Compositae (Sunflower family), 211
- Conductance of solutions of organosubstituted ammonium chlorides in liquid hydrogen sulfide, The, 60
- Confused flour beetle, *Tribolium confusum* Duval, toxicity studies on, 405
- Constants, elastic, determination of, by piezo-electric methods, 46
- Consumption, evaluations of, in modern thought, 95
- Cornstalks, anaerobic decomposition and gasification of, 233
- Coryneum, species of, 142

- Cyperaceae (Sedge family), 202  
*Cryptodi aporthe*, 129  
 Culture of fly larvae for use in maggot therapy, The, 81  
 Dairy products, micrococci isolated from, classification of, 43  
 Decomposition, an aerobic, of cornstalks by thermophiles, 233  
 Decomposition of lignin of plant materials, 11  
 Derivatives of 1-, 4-, 6-, and 9-substituted dibenzofurans, 92  
 Description of a dextro-lactic acid forming organism of the genus *Bacillus*, 187  
 Determination of elastic constants by piezo-electric methods, The, 46  
 Dextro-lactic acid, description of a, 187  
 Diaporthe, and related conidial forms, 129  
 Dibenzofurans, 92  
 Differentiability and continuity properties of solutions of certain partial differential equations of applied mathematics, 20  
 Differential, the general, operator, 261  
 Differentiation of the vitamins of the B complex and their distribution in certain foods, 103  
 Dinitro-o-cresol dusts, use of, in grasshopper control, 345  
 Dissimilation of levulose by heterofermentative lactic acid bacteria, the, 359  
 Dissimilation of phosphoglyceric acid and hexosediphosphate by *Aerobacter indologenes*, The, 253  
 Dosage-mortality relations toward confused flour beetles, *Tribolium confusum* Duval, 39  
 Dusts, value of, for potato insect control, 155
- Effect of decomposition of the lignin of plant materials, The, 11  
 Effect of ether on the toxicity of certain fumigants to the confused flour beetle, *Tribolium confusum*, Duval, 39, 405  
 Effect of growth of micro organisms on acid numbers of fat in cream and butter, 32  
 Effect of low temperatures on the intensity of fluorescence, 195  
 Effect of pH on the toxicity of nicotine injected into the cockroach, *Periplaneta americana* (L.), 305  
 Effect of quartz filters on the distribution of energy in laue patterns, 84  
 Effects of tomato juice on production of flavor contributants in butter cultures, 3  
 Effects of toxic compounds on the gustatory chemoreceptors in certain Diptera, 21  
 Effect of treated fats on vitamin A potency, 29  
 Efficacy of ultra-violet light sources in killing bacteria suspended in air, The, 215  
*Eimeria nieschulzi* growth-promoting potency of feeding stuffs, 317  
 Elastic constants, determination of, by piezo-electric methods, 46  
 Elastic theory, finite strain analysis in, 71  
 Elements, Group VIII, organometallic compounds of, 57  
 Energy, free, of soil water, 76  
 Equations, linear differential, 333  
 Equations, linear operational, use of functionals in obtaining approximate solutions of, 37  
 Equations, partial differential, properties of, 20  
 Erosion, effect of, in cultural treatment of plums, 389  
*Escherichia coli*, 216  
*Escherichia coli*, preparation of active juices from, 186  
 Ethanol, yield of, from various cereals, 78  
 Ether, effect of, on toxicity of fumigants to *Tribolium confusum*, 405  
 Euphorbiaceae (Spurge family), 209  
 Evaluations of consumption in modern thought, 95  
 Examination of ice cream with the Burri smear culture technic, 163
- Fats, effects of treated, on vitamin A potency, 29  
 Fatty acid derivatives, reactions of some high-molecular-weight, 64  
 Feeding stuffs, growth-promoting potency of, 317  
 Fermentation, butyl-acetonic, relation of structure of sugars to the chemism of, 40  
 Filters, quartz, effect of, on distribution of energy in laue patterns, 84  
 Finite strain analysis in elastic theory, 71  
 Flavor, in butter, 3  
 Fluorescence, effect of low temperature on intensity of, 195  
 Fly larvae, culture of, for use in maggot therapy, 81  
 Foods, distribution of vitamins in, 103



- Friedel-Crafts, reactions, 98  
 Fungi, estimating number in soil, 50  
 Fumigants, toxicity of, effect of ether on, 405  
 Functional methods, 333  
 Functional method for the solution of thin plate problems applied to a square, clamped plate with a central point load, 333  
 Functionals, use of, in obtaining approximate solutions of linear operational equations, 37  
 Fungi associated with tree cankers in Iowa. II *Diaporthe*, *Apioporthes*, *Cryptodiarporthe*, *Pseudovalsa* and their related conidial forms, 129  
  
 Gases, breakdown potentials of, under alternating voltages, 67  
 Gasification of cornstalks by thermophiles, 233  
 General differential operator, The, 261  
 Gramineae (Grass family), 200  
 Grasshoppers, control of, 345  
 Grasshoppers, reactions of, to castor bean plants, 353  
  
 Haloragidaceae (Water Milfoil family), 210  
 Heat, radiant, transfer in ceramic kilns, 48  
 Hemolymph, circulation of, in wings of *Blattella germanica* L., 107  
*Hemophilus influenzae avis*, study of, 73  
 Herbarium, Iowa State College, supplement to catalogue of Iowa plants in, 199  
 Heterofermentative lactic acid bacteria, dissimilation of levulose by, 359  
 Heterofermentative lactic acid bacteria, nutritive requirements of, 367  
 Hexosediphosphate, 253  
 Hillculture, practice of, 385  
 Hybrid and inbred strains of sweet corn, pericarpins, 379  
 Hydrogen sulfide, liquid, 60  
  
 Ice cream, examination of, with Burri smear culture technic, 163  
 Illecebraceae (Knotweed family), 207  
 Inbred strains of sweet corn, pericarp in, 379  
 Influenza, swine, studies on, 73  
 Insect, potato, control, 155  
 Insecticidal action of some substituted pyrrolidine, 53  
 Insecticidal action of substituted quinoline and tetrahydroquinolines, 69  
 Insecticides, 345  
 Insects as vectors of yellow dwarf, a virus disease of onions, 267  
 Ionization constants and insecticidal action of substituted quinolines and tetrahydroquinolines, 69  
 Iowa, fungi, 129  
 Iowa plants, supplement to catalogue of, in Iowa State College herbarium, 199  
  
 Juglandaceae (Walnut family), 205  
 Juice, active, preparation of, from bacteria, 179  
  
 Ketoses, functioning of, 364  
 Kilns, ceramic, 48  
  
 Lactic acid bacteria, 367  
*Lactobacillus buchneri*, 367  
*L. lycopersici*, 362, 367  
*L. manniitopoens*, 367  
 Larvae, fly, culture of, 81  
 Laue patterns, distribution of energy in, 84  
 Lauraceae (Laurel family), 207  
 Leafhoppers, 270  
 Leguminosae (Bean family), 209  
*Leuconostoc dextraniticus*, 362  
 Levulose, dissimilation of, 359  
 Levulose, dissimilation of, by heterofermentative lactic acid bacteria, 359  
 Light, ultra-violet, 215  
 Lignin, decomposition of, in plant materials, 11  
 Lobeliaceae (Lobelia family), 211  
  
 Maggot therapy, culture of fly larvae for use in, 81  
 Micrococci, proteolytic, classification of, isolated from dairy products, 43  
 Micro organisms, effect of growth of, on acid numbers of fat in cream and butter, 32  
 Moisture, content in soil, 76  
 Motility of the excised fore-gut of *Periplaneta americana* (Orthoptera) in various salt solutions, 393  
  
 Nabidae, new and unrecorded, 323  
 Najadaceae (Pondweed family), 200  
 Nature of *Eimeria nieschulzi* growth-promoting potency of feeding stuffs. I. Positive effect of gray shorts, 317  
 New Nabidae (Hemiptera), 323  
 Nicotine, toxicity of, injected into cockroach (*Periplaneta americana*), 309

- Nitrogen fixation by *Axotobacter*, 87  
 Nutritional role of zinc, 89  
 Nutritive requirements of the hetero-fermentative lactic acid bacteria, 367  
 Onagraceae (Evening Primrose family), 209  
 Onions, virus disease of, transmission of, 267  
 Oocysts of *Eimeria nieschulzi*, 320  
 Operator, general differential, 261  
 Organic compounds, association of, and electron-sharing ability of radicals, 55  
 Organisms, susceptibility of, to ultra-violet light, 228  
 Organometallic compounds of group VIII elements, 57  
 Organometallic radicals, 8  
 Organosubstituted ammonium chlorides in liquid hydrogen sulfide, 60  
 Orthotropic elastic layer, stresses in, 295  
 Oxidation of certain polyhydric alcohols by *Acetobacter suboxydans*, The, 24
- Pachynomus africanus*, n. sp. description of, 323  
 Pericarp, quantity of, in strains of sweet corn, 379  
*Periplaneta americana* (Orthoptera) motility of excised fore-gut of, in salt solutions, 393  
*Periplaneta americana* (L.), toxicity of nicotine when injected into, 309  
 Phenol compounds, 350  
 pH, Effect of, on toxicity of nicotine injected into cockroach, 305  
 Phomopsis, species of, 139  
*Phorticus abdominalis*, n. sp. description of, 325  
*Ph. flavus* var. *breviatus*, n. var., description of, 327  
*Ph. varicolor* Distant, 326  
 Phosphoglyceric acid, dissimilation of, and hexosodiphosphate by *Aerobacter indologenes*, 253  
 Physiological and nutritional role of zinc, 89  
 Plants, Iowa, in Iowa State College herbarium, 199  
 Plating method, for estimating number of bacteria in soil samples, 50  
 Polygonaceae (Buckwheat family), 206  
 Plum, response of, grown under hillculture conditions to modifications in cultural treatment, 385  
 Potato insect control, 155  
 Preliminary studies on the comparative value of some sprays and dusts in potato insect control, 155  
 Preliminary studies on the use of dinitro-o-cresol dusts in grasshopper control, 345  
 Preparation of an active juice from bacteria, 179  
 Preparation of cholesterilene and various cholestadienes, 101  
*Prostemma amysti* Reuter, 324  
*P. belidis*, n. sp., description of, 324  
 Proteolytic micrococci, classification of, isolated from dairy products, 43  
 Pseudovalsa, 129  
 Pyrrolidines, substituted, insecticidal action of some, 53
- Quantitative spectrographic analysis of soils, The, 35  
 Quantity of pericarp in several hybrid and inbred strains of sweet corn, The, 379  
 Quartz filters, effect of, on distribution of energy in laue patterns, 84  
 Quinolines, alpha substituted, 69
- Radiant heat transfer in ceramic kilns, 48  
 Radicals, electron-sharing ability of, 55  
 Radicals, organometallic, 8  
 Ranunculaceae (Crowfoot family), 207  
 Reactions of some high-molecular-weight fatty acid derivatives, 64  
 Relation between the free energy of soil water and the moisture content of the soil, 76  
 Relation between methods of saccharification and yield of ethanol from various cereals, The, 78  
 Relation of the structure of sugars to the chemism of the butyl-acetonic fermentation, 40  
 Relationship between the electron-sharing ability of radicals and the association of organic compounds, 55  
 Relationship of *Achromobacter putrefaciens* to the putrid defect of butter, 17  
 Response of the plum grown under hillculture conditions to modifications in cultural treatment, The, 385  
*Ricinus communis* L., as used for grasshopper control, 353  
 Rosaceae (Rose family), 207
- Saccharification and yield of ethanol from various cereals, 78  
 Salicaceae (Willow family), 204  
 Salt solutions, motility of fore-gut of

- Periplaneta americana* in, 393  
 Salts of zinc, 89  
 Santalaceae (Sandalwood family), 206  
*Sarcina lutea*, 217  
 Scrophulariaceae (Figwort family), 211  
 Soil, moisture content of, and free energy of soil water, 76  
 Soil, plating methods for estimating number of bacteria and fungi in, 50  
 Soils, quantitative spectrographic analysis of, 35  
 Some anomalous Friedel-Crafts reactions, 98  
 Some changes produced in growth, reproduction, blood and urine of rats by salts of zinc with certain observations on the effects of cadmium and beryllium salts, 89  
 Some factors influencing nitrogen fixation by *Azotobacter*, 87  
 Some new and heretofore unrecorded Nabidae (Hemiptera), 323  
 Some reactions of grasshoppers to castor bean plants, 353  
 Some studies in swine influenza  
   I. Comparative study of *Hemophilus influenzae avis* and *H. influenzae*  
   II. Antibody response to experimental swine influenza, 73  
 Sprays, and dusts, for potato insect control, 155  
 Strain, finite, analysis in elastic theory, 71  
*Staphylococcus aureus*, 217  
 Stresses in an orthotropic elastic layer, 295  
 Studies on the fermentative activity of yeast zymín, 27  
 Sugar, structure of, related to butyl-acetonic fermentation, 40  
 Sulphur dust, 157  
 Supplement to the catalogue of Iowa plants in the Iowa State College herbarium, The, 199  
 Swine influenza, studies on, 73  
  
 Temperature, effect of, on intensity of fluorescence, 195  
 Therapy, maggot, fly larvae in, 81  
  
 Thermophiles, anaerobic decomposition and gasification of cornstalks by, 233  
 Tetrahydroquinolines, alpha-substituted, 69  
 Thin plate problems, 333  
*Thrips tabaci* Lind., 269  
 Thyphaceae (Cat-tail family), 199  
 Toxic compounds, effects of, on gustatory chemoreceptors in certain Diptera, 22  
 Toxicity of fumigants, effect of ether on, 405  
 Toxicity of nicotine, effect of pH on, injected into the cockroach, 305  
 Tree cankers in Iowa, fungi associated with, 129  
*Tribolium confusum* Duval, effect of ether on toxicity of fumigants to, 405  
   *T. confusum* Duval, toxicity studies with, 39  
  
 Ultra-violet light, effectiveness in killing bacteria, 215  
 Use of functionals in obtaining approximate solutions of linear operational equations, 37  
  
 Vectors, insects as, of a virus disease of onions, 267  
 Violaceae (Violet family), 210  
 Virus disease of onions, yellow dwarf, 267  
 Vitamin A, potency of, effect of treated fats on, 29  
 Vitamin B complex, 103  
 Vitamins, differentiation of, of the B complex, 103  
 Voltages, alternating, breakdown potentials of gases under, 67  
  
 Welfare, as affected by consumption, 95  
  
 Yeast zymín, fermentative activity of, 27  
 Yellow dwarf, vectors of, 267  
  
 Zinc, physiological and nutritional role of, 89  
 Zymín, yeast, fermentative activity of, 27











